# Infection characteristics of *Salmonella enterica* serovar Brandenburg *in vitro*

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A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Master of Applied Science

at

Lincoln University

by

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Lincoln University

2011

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Applied Science.

# Infection characteristics of *Salmonella enterica* serovar Brandenburg *in vitro*

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*Salmonella enterica* serovar Brandenburg (*S.* Brandenburg) is primarily recognized as a pathogenic agent causing acute diarrhoea in humans and livestock. In New Zealand, it also causes abortion and septicaemia, particularly in pregnant ewes. The disease outcome and severity may depend on serovar- and host-specific factors. In this study, the uptake and persistence characteristics (adhesion, invasion and replication) of selected *S.* Brandenburg isolates were investigated, using primary cell cultures derived from ovine and bovine oviduct and intestine, in an attempt to help explain why the agent is so pathogenic in sheep.

In the first study, different profile 14 (epidemic) *S.* Brandenburg isolates 3684, 3062 and 4468 and non-profile 14 *S.* Brandenburg isolate 4527 were added into *ex vivo* and *in vitro* cell cultures derived from sheep oviduct. In three independent assays adhesion, invasion and replication were measured at 1, 2, and 24 h respectively. In the second study, *ex vivo* or *in vitro* cell cultures from sheep oviduct were infected with either *S.* Brandenburg isolate 3684 or *S.* Typhimurium isolate 1979 and adhesion, invasion and replication characteristics were compared. In the third study, *S.* Brandenburg isolate 3684 was added to *ex vivo* and *in vitro* the ovine oviduct epithelial cell (OOEC) or bovine oviduct epithelial cell (BOEC) cultures and same time course measurements were made. In the fourth study, *S.* Brandenburg isolate 3684 was added to *in vitro* cell cultures derived from either ovine intestine cells (OIECs) or ovine oviduct cells (OOECs) and the same time course measurement were compared. Bacterial counts were estimated after 10 fold serial dilution onto LB agar plates and overnight incubation at 37$^{o}$C. The adhesive, invasive and replicative characteristics of the different *Salmonella* isolates were compared using a Student two-sample *t*-test.

*S.* Brandenburg profile 14 isolates 3062, 3684, 4468 shared similar adhesive, invasive and replicative capabilities in both *ex vivo* and *in vitro* OOECs. Invasion of non-profile 14 isolate

4527 was less than profile 14 isolates 3684, 3062 and 4468 in both *ex vivo* and *in vitro* OOECs ($P<0.01$). Secondly, *S.* Brandenburg isolate 3684 more readily adhered to, and replicated within, OOECs than *S.* Typhimurium isolate 1979, in *ex vivo* OOECs ($P<0.01$). Thirdly, *S.* Brandenburg isolate 3684 more readily replicated within OOECs ($P<0.01$) than BOECs in both *ex vivo* and *in vitro* assays. Fourthly, *S.* Brandenburg isolate 3684 more readily invaded and replicated within *in vitro* OOECs than OIECs ($P<0.01$).

Together, these results suggest that *S.* Brandenburg profile 14 isolates share some phenotypic characteristics regarding infection and suggest that virulence of *S.* Brandenburg *in vivo* is associated with its infectivity characteristics *in vitro* and *ex vivo*. In addition, the characteristics of *S.* Brandenburg field infections in sheep may be due to its preference for infecting *in vitro* OOECs, not BOECs or OIECs.

**Keywords**: *S.* Brandenburg, pregnant ewes, abortion, adhesion, invasion, replication, ovine oviduct epithelial cells (OOECs), bovine oviduct epithelial cells (BOECs), ovine intestinal epithelial cells (OIECs).

# Acknowledgements

I would like to thank the following people who have kindly helped and guided me throughout my Master study and during the writing of my thesis. Grateful thanks are given to my supervisor Dr. Robin McFarlane for his supervision, enthusiasm, corrections and constructive criticism from the start to the end of my study. Special thanks also to my associate supervisor Dr Graham Kay for his advice and encouragement in cell culture. I would like to thank Dr. Susan Paulin for her professional assistance with the infection assay and for supply of the *Salmonella* strains. Special thanks go to Omega Amoafo for his kind assistance in the laboratory. I am thankful to Richard Sedcole for his help with statistical analysis and Malvern Homekill Services for supplying tissues. Finally, I am indebted to my father Minggui Piao and my mother Ying Liu for their support, encouragement and understanding. And a very special love to my partner Maggie Hong for bringing joy, relaxation and comfort.

# Table of Contents

# List of Tables

# List of Figures

# List of Abbreviations

| | |
|---|---|
| °C | Degree Celsius |
| µl | Microliter |
| µm | Micrometer |
| APCs | Antigen presenting cells |
| BOEC | Bovine oviduct epithelial cells |
| BSA | Bovine serum albumin |
| CFUs | Colony-forming units |
| CMI | Cell-mediated immunity |
| $CO_2$ | Carbon dioxide |
| DCs | Dendritic cells |
| D-MEM | Dulbecco's Modified Eagle Medium |
| DNA | Deoxyribonucleic acid |
| D-PBS | Dulbecco's phosphate-buffered saline |
| EGF | Epidermal growth factor |
| FAE | Follicle-associated epithelium |
| FBS | Foetal bovine serum |
| FITC | Fluorescein isothiocyanate |
| g | Gauge |
| *g* | Gravity |
| GALT | Gut-associated lymphoid tissue |
| GIT | Gastrointestinal tract |
| h | Hour(s) |
| H&E | Haematoxylin and Eosin |
| $H_2O$ | Water |
| IFN-γ | Interferon gamma |
| Ig | Immunoglobulin |
| IL | Interleukin |
| iNOS | Nitric oxide synthase |
| LB | Luria-Bertani |
| LPS | Lipopolysaccharide |
| M | Molar |
| M cells | Microfold cells |
| MALT | Mucosa-associated lymphoid tissue |
| MHC | Major histocompatibility complex |
| min | Minute(s) |
| ml | Milliliter |
| MLEE | Multilocus enzyme electrophoresis |
| mM | Millimolar |
| mm | Millimeter |
| MOI | Multiplicity of Infection |

| | |
|---|---|
| MRFLP | Macro-restriction fragment length polymorphism |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NK | Natural killer |
| NKT | Natural killer T |
| NRAMP1 | Natural-resistance-associated macrophage protein 1 |
| $O_2$ | Oxygen |
| OD | Optical density |
| OIEC | Ovine intestinal epithelial cells |
| OMPs | Outer membrane proteins |
| OOEC | Ovine oviduct epithelial cell |
| PAMP | Pathogen-associated molecular pattern |
| Pen/Strep | Penicillin/streptomycin |
| PI | Pathogenicty islands |
| RES | Reticuloendothelial system |
| PFGE | Pulsed field gel electrophoresis |
| RNI | Reactive nitrogen intermediate |
| ROI | Reactive oxygen intermediate |
| SCV | *Salmonella* containing vacuole |
| Sifs | *Salmonella*-induced filaments |
| S-IgA | Secretory IgA |
| SPI-1 | *Salmonella* pathogenicity island 1 |
| SPI-2 | *Salmonella* pathogenicity island 2 |
| T3SS | Type III secretion system |
| TLRs | Toll-like receptors |
| TNFα | Tumor necrosis factor α |
| v/v | Volume per volume |
| w/v | Weight per volume |

# Chapter 1
# Introduction

During the 1990s, *Salmonella enterica* serovar Brandenburg (*S.* Brandenburg) was recognized as a causative pathogen of late pregnancy abortion and death amongst sheep in New Zealand. One year later, the organism had spread into dairy cattle and beef cattle herds, causing diarrhoea, dysentery, abortion and death with a lower prevalence and less commonly also in other species such as horse, dogs, goats, pigs and deer (Clark, et al., 2000; Higgan, et al., 2001). An average lambing loss of 17% on affected farms was recorded throughout the southern region of the South Island of New Zealand during the disease peak between 2000 and 2005 (Boxall, et al., 1999).

Ingestion of *Salmonella* is one of the predominant entry points for subsequent illness (Tannock & Smith, 1971). *Salmonella* express a range of mechanisms to survive and pass through the host gut, such as tolerance to the low pH conditions in the stomach, resistance to the components of digestion and competition with established microorganisms allowing penetration and colonization (Baumler, et al., 2000). *Salmonella* may reach the small intestine, penetrate the intestinal wall, multiply in the gut-associated lymphoid tissue (GALT) of the sub-mucosa and Peyer's patches (Carter & Collins, 1974; Samuel, 1981) and trigger mucosal inflammation and diarrhoea. Some serovars such as *S.* Typhimurium are confined to the intestine and only damage the alimentary tract whereas others such as *S.* Brandenburg can migrate into the mesenteric lymph nodes, spread via the efferent lymphatic vessel through the thoracic duct into the blood stream and be carried further to the liver, spleen and reproductive tract, eventually causing abortion in sheep and cattle (Baumler, et al., 2000).

The mechanism by which the serovar Brandenburg causes abortion in sheep and cattle in New Zealand still remains unknown. However, the infection characteristics of the microorganisms including adhesion, invasion, survival and replication may play important roles in the process of infection and may influence reproductive outcomes.

The literature review firstly describes the disease caused by *S.* Brandenburg. Secondly, the characteristics of *Salmonella* such as taxonomy, antigenic specificity, biochemical properties and genetic basis to virulence will be reviewed. Thirdly, the mechanisms of innate and specific response to *Salmonella* will be compared.

While important findings revealing the interaction between *S.* Brandenburg and sheep have been documented in recent years, the mechanism of abortion caused by *S.* Brandenburg is still poorly understood. Recent New Zealand research on *S.* Brandenburg has focused on the comparison of genomic sequences from *S.* Typhimurium and *S.* Brandenburg to identify potential genes responsible for *S.* Brandenburg virulence (Brandt, et al., 2008). There remains a need to understand the mechanisms underlying host specificity for *S.* Brandenburg and why does *S.* Brandenburg but not *S.* Typhimurium typically cause reproductive disease in New Zealand.

Therefore, this research had four hypotheses:

1. That *S.* Brandenburg isolates differ in their infective abilities for ovine oviduct epithelial cells.

2. That ovine oviduct epithelial cells are more 'susceptible' to infection by *S.* Brandenburg than *S.* Typhimurium.

3. That ovine oviduct epithelial cells are more 'susceptible' to infection with *S.* Brandenburg than bovine oviduct epithelial cells.

4. That ovine oviduct epithelial cells are more 'susceptible' to infection by *S.* Brandenburg than ovine intestinal epithelial cells.

These research objectives would be addressed by assessing the infection characteristics of *Salmonella* such as adhesion, invasion and replication in primary cultures of oviduct or intestinal epithelial cells.

# Chapter 2
# Literature review

## 2.1 Historical prevalence of *S.* Brandenburg

*S.* Brandenburg is a Gram negative, facultative intracellular bacterium which has been recognized as causative of an emerging zoonosis in New Zealand. In the early 1930s, the bacterium was first isolated from a human case in a town in Germany called Brandenburg (Kauffmann & Mtsui, 1930). In New Zealand, *S.* Brandenburg was first isolated from a sewage swab in 1966 and subsequently from cases of human gastroenteritis (Clark, et al., 2004). In 1996, *S.* Brandenburg was isolated from an aborted foetus in a Merino ewe in Canterbury (Bailey, 1997). Soon after that the disease was recognized as a causative pathogen of late pregnancy abortions and deaths amongst sheep in the South Island of New Zealand. One year later, the organism had spread into dairy cattle and beef cattle herds and less commonly in other species such as horses, dogs, goats, pigs and deer, causing diarrhoea, dysentery, abortion and death (Clark, et al., 2000; Higgan, et al., 2001).

Infections due to *S.* Brandenburg can be found worldwide, but it has been most common in the developed countries, such as Germany (Kauffmann & Mtsui, 1930), England (Jones, et al., 1964) Switzerland (Baquar, et al., 1994), France (Lamisse, et al., 1971), Spain (Curbelo, et al., 1954), Italy (Scuderi, et al., 2000), Belgium (van Looveren, et al., 2001), Hungary (Rowe, 1987), Australia (Murray & Davos, 1995), New Zealand (Bailey, 1997) and Japan (Hamada & Tsuji, 2001).

### 2.1.1 Human *S.* Brandenburg infections in New Zealand

In New Zealand, *S.* Brandenburg has historically been an uncommon cause in human infections, and has accounted for only 142 (1%) of the 14,000 salmonellosis case isolates serotyped between 1985 and 1994. From 1985 to 1989 there were 68 viable human isolates of *S.* Brandenburg and 74 cases between 1990 and 1994 (Baker, et al., 2007; Wright, et al., 1998).

However between 1995 and 2001, non-typhoidal salmonellosis had increased in New Zealand, peaking at 2417 cases in 2001 of which 137 cases were due to *S.* Brandenburg, with

5.1% of the cases occurring in the Auckland-Waikato region and 69% in the Otago-Southland region.

From 2002 to 2010, the number of salmonellosis cases due to *S.* Brandenburg in humans decreased to an average 57 human cases per year (Figure 2.1).

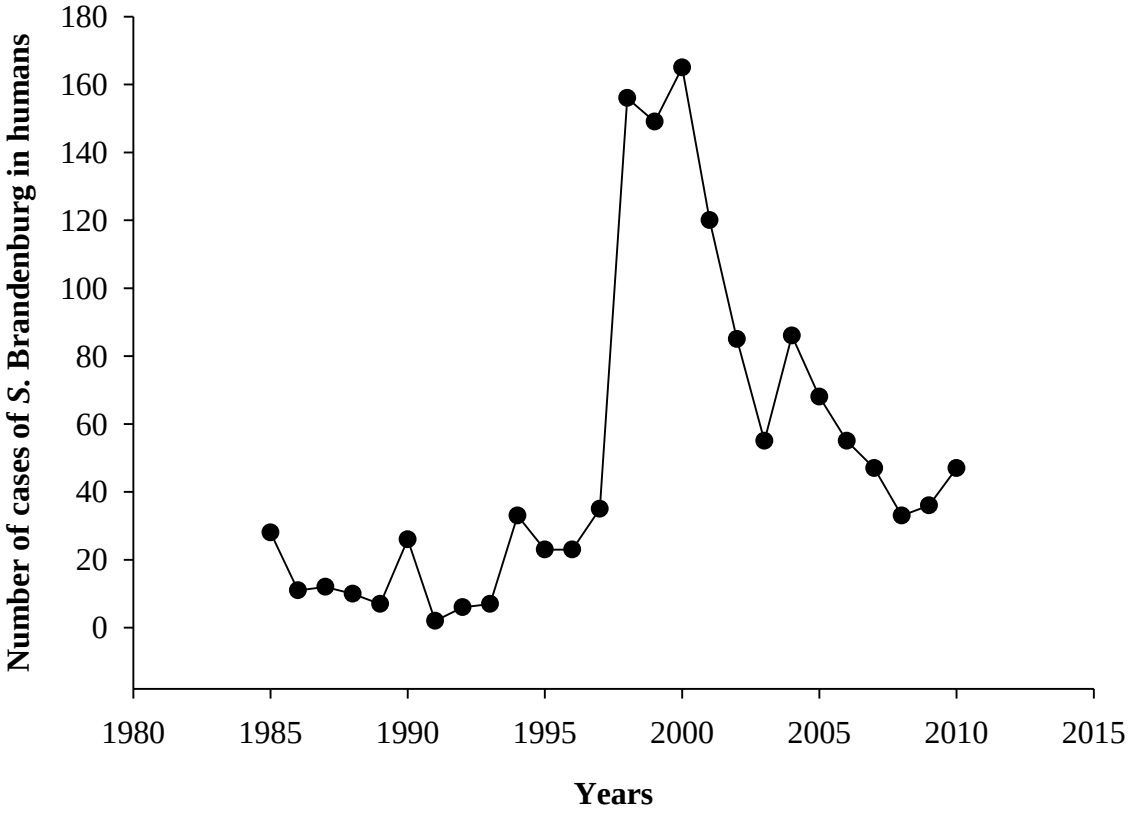



**Figure 2.1 Incidence of human infection in New Zealand due to *S.* Brandenburg (Thornley, 2002; Wright, et al., 1998).**

### 2.1.2 Non-human *S.* Brandenburg infections in New Zealand

Prior to 1996, *S.* Brandenburg was rarely found in non-human sources (Wright, et al., 1998). In 1996, *S.* Brandenburg was recorded from a foetus aborted from a sheep in Canterbury, New Zealand (Bailey, 1997). Subsequently, in 1998 the microorganism caused outbreaks of abortion and death in sheep in Mid- and South Canterbury, South- and West Otago, and Southland (Boxall, et al., 1999; Clark, et al., 2000; Roe, 1999). One year later, the organisms had spread into dairy and beef cattle herds, causing diarrhea and dysentery in adult cattle and calves, abortions and deaths in first calvers, and to a lesser extent in second calvers (Clark, 2001; Clark, et al., 2000). The epidemic curve in sheep and cattle in New Zealand illustrates the lower prevalence of *S.* Brandenburg in cattle compared with that in sheep (Figure 2.2).

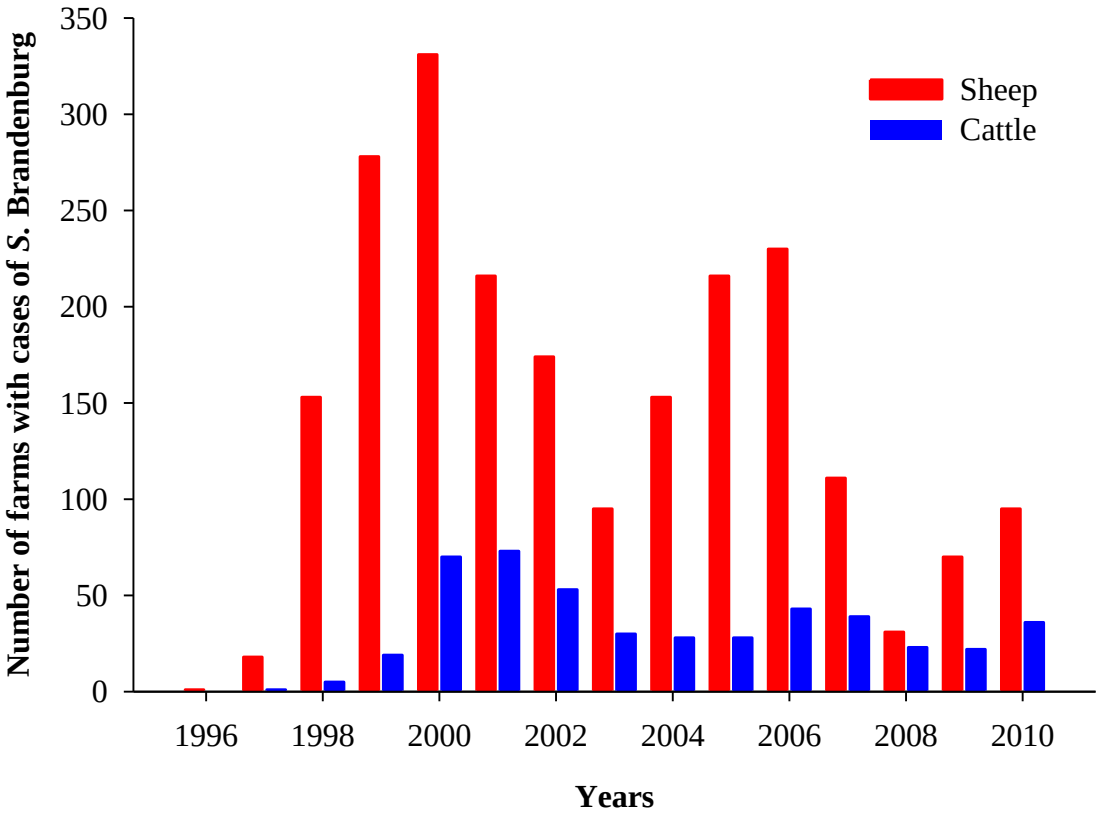



**Figure 2.2 Number of New Zealand farms with disease caused by *S.* Brandenburg (Clark, 2001).**

The majority of cases of infection with *S.* Brandenburg occurred in the winter or spring period and primarily in pregnant ewes. Data from farms that had harboured *S.* Brandenburg identified an abortion prevalence of 6%-20% in two-tooth ewes and 3% in mixed-aged ewes. The fatality rate following abortion in the pregnant ewes was typically around 50%, but

reached 100% on certain farms (Clark, et al., 2004). In the field, *S.* Brandenburg is less virulent in non-pregnant ewes with a smaller number of deaths recorded following clinical signs such as diarrhoea, and dysentery but at high experimental doses is lethal (Li, et al., 2005).

## 2.2 Transmission

Although it is still not clear how and where *S.* Brandenburg originated in New Zealand, subsequent spreading of these bacteria by environment contamination has been considered to play a critical role in prevalence of the salmonellosis due to it today. The primary infective pathway for spread is believed to be ingestion of microorganisms and subsequent colonization in the gastrointestinal tract (GIT) (Clark, et al., 2000). Furthermore, sheep can be also infected via inhalation of low doses of *Salmonella* organisms (Tannock & Smith, 1971). Since asymptomatic sheep have been shown to excrete *S.* Brandenburg in faeces for up to 6 months (Li, et al., 2005), contamination of the environment continues for a long time. The organisms can also spread in aborted foeti placenta and uterine discharges excreted by infected animals (Hunter, 1990). Epidemiological surveys showed that wild scavenging birds such as black-backed gulls (*Larus dominicanus*) are likely to be vectors of salmonellosis following ingestion of aborted foetuses or dead ewes (Clark, et al., 2004). It was found that *S.* Montevideo (Coulson, et al., 1983) and *S.* Brandenburg (Clark, et al., 2004) could multiply in the intestinal contents of gulls and were shed at high levels in their faeces. Farm workers or equipment may act as fomites for the transfer of *Salmonella*; similarly healthy persons infected with *Salmonella* may be carriers for the bacteria (Kotova, et al., 1988).

## 2.3 Clinical signs and pathological lesions of *Salmonella* Brandenburg infections

Pathology caused by *S.* Brandenburg varies amongst different hosts. In humans, *S.* Brandenburg is usually restricted to gastroenteric disease, which will lead to typical clinical signs such as severe diarrhoea, abdominal pain, stomach cramps and fever, which may last for up to six weeks (Clark, et al., 2004). However, *S.* Brandenburg has also been reported as a pathogen of extra-intestinal diseases such as a thigh abscess in Sweden (Bjorkman, et al., 2002), suppurative thyroiditis and abscess of ovaries in Italy (Chiovato, et al., 1993; Magliulo, 1982), peritonitis and septicaemia in France (Lamisse, et al., 1971; Laurens, et al., 1991), aortic aneurysm and manubriosternal junction osteomyelitis in the United Kingdom (Bliss, et al., 1968; Chattopadhyay, et al., 1990) and urinary tract infection in Germany (Adam &

Daschner, 1973). In New Zealand, extra-intestinal diseases due to infections with *S.* Brandenburg in human have included pericarditis, bacteraemia and pneumonia (Clarke & Tomlinson, 2004).

In animals, infections due to *S.* Brandenburg typically cause gastroenteric disease. Diarrhoea is one of the most common clinical signs among infected animals. In addition to the intestinal disorders, in areas of the South Island of New Zealand, *S.* Brandenburg has caused abortion and often subsequent death in pregnant ewes but it is less common in cattle. *S.* Brandenburg infection is common during the winter-spring season which is different from other *Salmonella* serovars (Robinson, 1970). The infection usually affects pregnant ewes from around the $80^{th}$ day of gestation, peaking about 100-120 days gestation (Clark, et al., 1999; Smart, 2000). Experimentally infected ewes given 10.3 $\log_{10}$ CFUs become depressed and anorexic after 2 days and especially those with multiple foeti may abort and die (Li, et al., 2005). In peracute cases the uterus of ewes will enlarge and darken (Clark, et al., 2007) and the expelled foetuses commonly have a 'cooked' appearance with swollen liver, blood tinged stomach contents, oedematous subcutaneous tissue and characteristic putrid smell (Figure 2.3).

Histological examination of placental samples showed that the capillaries of the placenta were packed with numerous *S.* Brandenburg that might trigger inflammatory reactions in the placental chorion, resulting in dysfunction of placenta and abortion or death of the foetus (Clark, et al., 1999).

**Figure 2.3 Abortion and death caused by *S.* Brandenburg (Clark, et al., 2004).**

## 2.4 Disease control

Several approaches are recommended with regard to controlling the transmission of salmonellosis, including the use of vaccine, antibiotic treatment and changes in farm management.

Salvexin+B, a vaccine that is commercially available in New Zealand and produced by Intervet/Schering-Plough New Zealand contains antigens from the four most common serovars of *Salmonella* (i.e. *S.* Typhimurium, *S.* Bovismorbificans, *S.* Hindmarsh and *S.* Brandenburg) (Marchant, 2000). Even though a such vaccine can reduce the incidence of abortion and death caused by *S.* Brandenburg, only partial protection is evident under high challenge (Kerslake & Perkins, 2006; Li, et al., 2005) and is not in common use even in regions where the disease is present (Hicks, 2006). A recent study reported that a semi-purified subunit vaccine is likely to be superior to this commercial available vaccine, at least with experimental infections (Li, et al., 2005).

Antibiotic treatment is another approach for sheep farmers to protect sheep stock from an outbreak of this disease. Antibiotic sensitivity testing in sheep showed that *S.* Brandenburg is sensitive to many antimicrobial drugs including ampicillin, cephalothin, enrofloxacin, streptomycin, tetracycline, ciprofloxacin, nalidixic acid and trimethoprim, but resistant to lincomycin and penicillin (Clark, et al., 2004; Wybot, et al., 2004). Mass treatment of flocks has been attempted successfully in the field (Smart, 2000). However, the disadvantage of using antibiotics widely is the increasing risk of developing new strains which are multi-drug-resistant (Poppe, et al., 1998).

A change in farm management may reduce the risk of occurrence and severity of the disease. There are a number of preventative and control measurements, such as, rapid disposal of aborted foetuses, isolation of aborted ewes, reduction in stress levels for pregnant ewes, control of scavengers, cleaning and disinfection of vehicles and care in buying sheep (Clark, et al., 1999). Break feeding (or strip grazing) is a common grazing system for sheep in New Zealand. Such a method keeps large numbers of stock on a small area with an electric fence and as a result high levels of environmental contamination with *S.* Brandenburg occur (Kerslake & Perkins, 2006). Destocking or allowing animals to graze over larger areas reduces the risk of disease.

## 2.5 Characteristics of *Salmonella*

### 2.5.1 Taxonomy of *Salmonella*

The taxonomy of *Salmonella* is complex and has been revised several times over the years (Kauffmann & Edwards, 2005; Tindall, et al., 2005). At present, the genus consists of three species, which are *S. bongori*, the recently identified *S. subterranea* (Shelobolina, et al., 2004) and *S. enterica* as described by Baumler (1997) (Figure 2.4). *S. enterica* can be subdivided into six subspecies that are subdivided into serovars according to their classical antigenic composition. *S. enterica* ssp. *enterica* (I) includes most clinically relevant serovars accounting for 99% of all known salmonellosis in warm blooded hosts. One example is *S.* Brandenburg that can be described in this group as *Salmonella enterica* serovar Brandenburg or *Salmonella enterica* subsp. *enterica* serovar Brandenburg. Identification of *Salmonella* subspecies are also based on antigenic specificities, biochemical properties and genetic analysis (Le Minor & Popoff, 1987).

### 2.5.2 Antigenic specificities

*Salmonella* possesses three main antigens: flagellar antigen (H) somatic antigen (O) and capsular or virulence antigen (VI). Serological analysis of O and H antigens was initiated by White (1926) and extended by Kauffmann (1966). The H antigen is heat labile and contains proteinaceous flagellins. The O antigen is a heat stable antigen which is composed of phospholipid-polysacharide complexes (Kauffmann, 1966). While the VI antigen is a capsular polysaccharide overlying the O antigen, it is present in only a few serovars, e.g. *S.* Typhi and S. paratyphi C (Felix & Pitt, 1936).

The subspecies are divided into over 50 serogroups based on somatic (O) antigens present. The serogroups are further divided into over 2300 serotypes based on flagellar (H) antigens. Currently, there are approximately 2400 specific serovars that have been identified, based on the Kauffmann-White antigenic scheme. These organisms are recorded by genus and serotype (or serogroup). The serotype formula for *S.* Brandenburg is somatic (O) antigens 1,4, [5], 12, 27 and flagella (H) antigens phase 1:1, v and phase 2:e, n, z15 (Bjorkman, et al., 2002). This is similar to the somatic antigens of *S.* Typhimurium (O antigen: 1,4,5, 12) (McWhorter-Murlin & Hickman-Brenner, 1994).

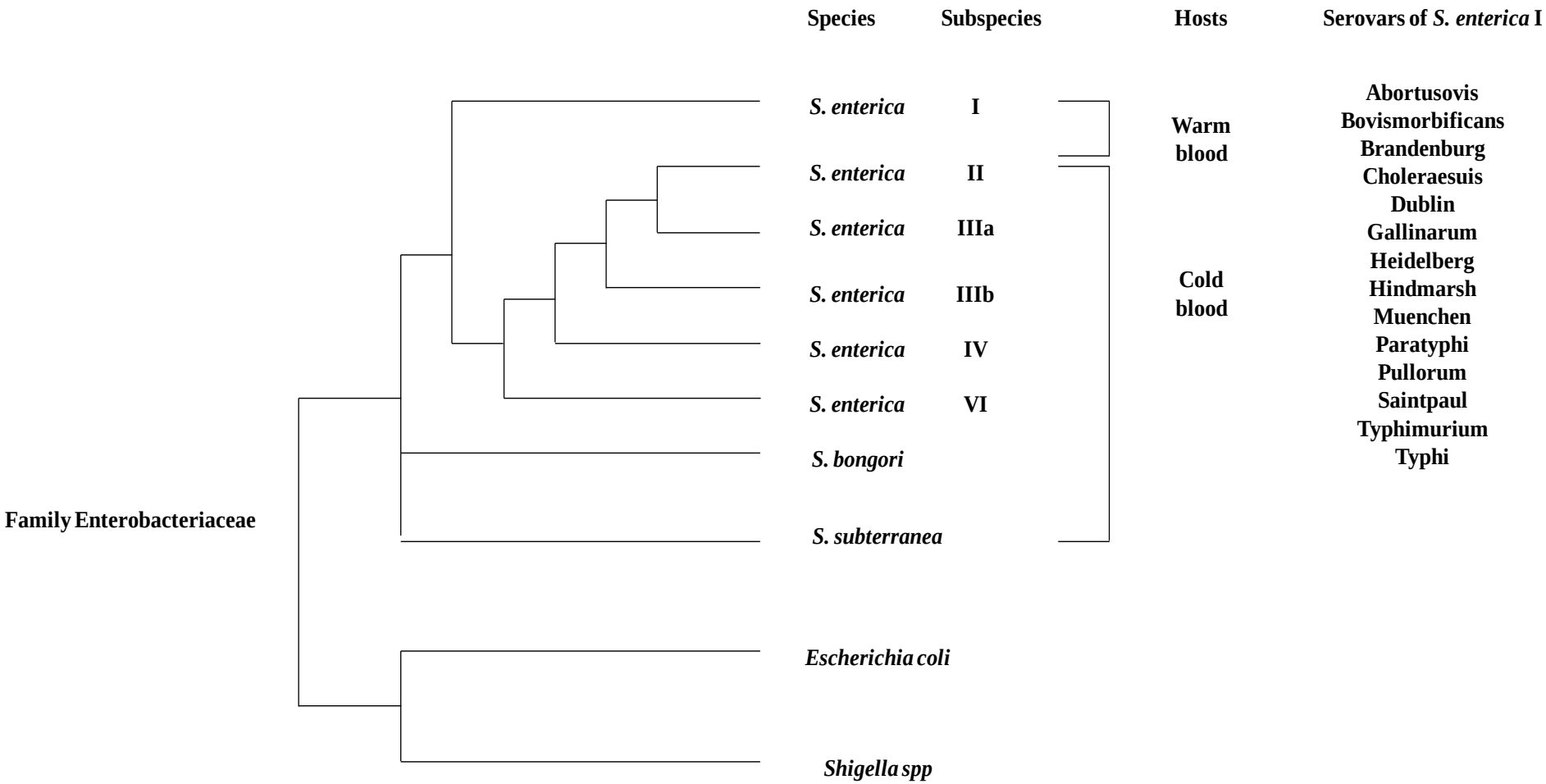



**Figure 2.4 Dendrogram of *Salmonella* classification (Baumler, 1997).**

### 2.5.3 Biochemical properties

Strains of *S. enterica* have the following properties which are typical of the family enterobacteriaceae. *Salmonella enterica* do not produce indole or ferment glucose by the mixed acid fermentation. Unlike other serovars, *S.* Brandenburg does not produce phenylalanine deaminase or urease, but it does utilize citrate as sole carbon source, and produce hydrogen sulfide ($H_2S$). It is motile and ferments a variety of carbohydrates and decarboxylate arginine, lysine and/or ornithine (Le Minor, et al., 1970; Le Minor & Popoff, 1987).

### 2.5.4 Genotypic analysis

A bacterial species can be defined as a DNA hybridization group. Strains within a species are generally more than 70% related (Wayne, et al., 1987). DNA hybridization studies (Crosa, et al., 1973; Le Minor, et al., 1982; Stoleru, et al., 1976) have classified the genus *Salmonella* into only three genomic species, *S. enteric, S. bongori and S. subterranean* (see 2.5.1).

Furthermore, the multilocus enzyme electrophoresis (MLEE) method has been used to assess allelic variation in multiple genes among *Salmonella* isolates. This analysis indicates that *S. bongori* is the most divergent group of *Salmonella*. In *S. enterica* subsp. *enterica*, MLEE analysis shows serovar Typhi as a single clone, distinct from all other serovars studied. Serovars Paratyphi A and Sendai are in a group, whereas serovars Typhimurium, Paratyphi B, Saintpaul, Heidelberg and Muenchen harbour a loose cluster. MLEE analysis has identified serovar Enteritidis as a close relative of serovar Gallinarum (Selander, et al., 1996). Aside from MLEE, pulsed field gel electrophoresis (PFGE) was used to type *S.* Brandenburg isolates in New Zealand. Macro-restriction fragment length polymorphism (MRFLP) analysis revealed 13 different patterns typed from human isolates recovered between 1990 and 1995 (Wright, et al., 1998). In 1999, a different but identical molecular pattern was seen in all isolates from 14 sheep, 19 cattle and one dog. In 2000, 45 sheep-yard dust isolates were typed and, with one exception, all had the same molecular pattern termed profile 14. The DNA pattern from the exceptional isolate provided the first evidence of diversion from the *S.* Brandenburg sheep abortion epidemic strain (Clark, et al., 2003).

## 2.6 Virulence mechanisms of *Salmonella* and infection progression

The basic virulence strategy of *Salmonella* is complicated, which involves several critical stages in terms of crossing the epithelial lining of the small intestine, reaching the blood

stream, disseminating further into the reticuloendothelial system of the host, inducing macrophage apoptosis and host death.

*Salmonella* are enteroinvasive bacteria that typically gain entry to the gastrointestinal tract via contaminated food or water; most bacteria are killed in the stomach or passed out of gut. The alimentary tract is a hostile environment, which imposes multiple stresses upon invading bacteria. The first host defence is the acid barrier of the stomach for oral infections (Bearson, et al., 1997). Although this acid environment inactivates many bacteria, *Salmonella* may survive. For example, Carter and Collins (1974) found that 1% of *S.* Enteritidis inoculum can survive in a low pH environment during the passage through the stomach.

Within the small intestine, *Salmonella* encounter the action of bile salts which are detergents made by the liver and secreted into and stored in high concentrations in the gallbladder. Bile-containing salts are released into the intestine to aid in the dispersion and degradation of fats. Enteric bacteria, including *Salmonella*, are resistant to the effects of bile, a finding that has been used clinically in the selective enrichment of these organisms (van Velkinburgh & Gunn, 1999). Like other enterobacteria, some *Salmonella* serovars are well adapted to cope with this stress condition and gain a foothold at the preferred niche in the intestinal wall, the gut-associate lymphoid tissue (GALT). For example, it was found that during infection of mice with *S.* enteritidis, about 5% of the bacteria that survive the passage through the stomach manage to penetrate the intestinal wall of the small intestine and reach the GALT (Carter & Collins, 1974).

The indigenous gut flora is another important factor that can inhibit colonization by these serovars; known as bacterial interference. Several mechanisms of bacterial interference have been proposed, including production of inhibitory substances, competition for tissue adhesion sites and limiting of nutrients. The primary site of intestinal invasion is the distal ileum where some *Salmonella* effector proteins are involved in translocation into host cells to drive their own uptake. In mammals, lymph follicles are clustered as Peyer's patch typically where *Salmonella* enter.

Follicle-associated epithelium (FAE) overlying Peyer's patches contains microfold cells (M cells) (Figure 2.6) which are not found in villous epithelium (Giannasca, et al., 1994; Pappo & Owen, 1988).

Although M cells are involved in maintaining immune response at the different sites of MALT, numerous bacteria such as *Salmonella* selectively adhere to, and efficiently pass

through, both enterocytes and M cells enriched within the FAE, as well as other epithelial cells (Mastroeni & Sheppard, 2004). For example, at an early stage of infection with *S.* Typhimurium, it has been noted that large membrane ruffles appear on the apical surface of the M cell and, within a short period of time (30-60 min), the cell becomes necrotic and begins to die (Clark, et al., 1994) (Figure 2.6). M cells are usually surrounded by lymphocytes and this association provides a direct interaction for antigen presentation. An alternative mechanism of invasion, independent of M cells, is where *Salmonella* is engulfed by dendritic cells (DCs) that open the tight junctions between epithelial cells through tight-junction proteins and directly sample bacteria (Rescigno, et al., 2001).

Having traversed the intestinal mucosa, *Salmonella* drains to the regional lymph nodes, where macrophages that line the lymphatic sinuses form an effective barrier to prevent further spread (Figure 2.5). If the bacterial extension is limited at the regional lymph nodes, infection remains within the intestine and only causes localized disease with diarrhea, a typical sign of disease (Baumler, et al., 2000). On the other hand, if the macrophages draining the lymph nodes are not able to limit spread, systemic disease can ensue. During systemic infection, the pathogens spread via the efferent lymphatics and the thoracic duct into the vena cava and eventually seed the filtering organs of the reticuloendothelial system (RES)-liver and spleen. A microscopic examination revealed that in liver, the initial lesions appeared at early stage of infection with masses of infiltrating neutrophils accumulated in well defined necrotic foci. Macrophages began to appear in the periphery of lesions in the following days, replacing the neutrophils, since neutrophils are short-lived (Conlan, 1997). During the late stage of infection the macrophages were not only present in the foci, but also in large numbers in the interstitial area of the parenchyma causing leucostasis of the liver. Similarly in spleen, the majority of infected splenocytes are surrounded by neutrophils at an early stage of infection. However; *Salmonella* are localized to specific subsets of marginal zone and red pulp macrophages at the late stage of infection (Salcedo, et al., 2001; Thone, et al., 2007). It is clear that massive numbers of *Salmonella* organisms are phagocytosed by macrophages and DCs. However, rather than being destroyed by these phagocytes upon engulfment, *Salmonella* has evolved several mechanisms to survive in the harsh milieu of phagosomal compartments (Foster & Spector, 1995). For example, Rathman et al. (1997) found that approximate 75% of *S.* Typhimurium can bypass the fusion of phagosomes with lysosomes by diverging from the normal endocytic trafficking pathway of the host cells. Although, very little information upon the mechanism can be obtained, it has been widely accepted that *Salmonella* can survive and

even replicate within macrophage phagososmes and be cytotoxic to macrophages by inducing apoptosis *in vitro* (Chen, et al., 1996; Maw & Meynell, 1968; Monack, et al., 1996).

The cytotoxicity manifests itself in at least two different ways: initial impairment of the ruffling and macro-pinocytic activities of the infected macrophages in which the infected macrophage begin to 'round followed by death of the infected macrophages. During this time, *Salmonella* organisms exhibited several features of apoptotic cells such as membrane blebbing, typical chromatin condensation and fragmentation with the presence of apoptotic bodies (Chen, et al., 1996). Cytotoxic effects are dependent upon the expression of the T3SS (See section 2.7.5). At the end of this phase, bacteria reappear in the blood and replicate rapidly until the animal dies. Death may result from endotoxic shock (Khan, et al., 1998).

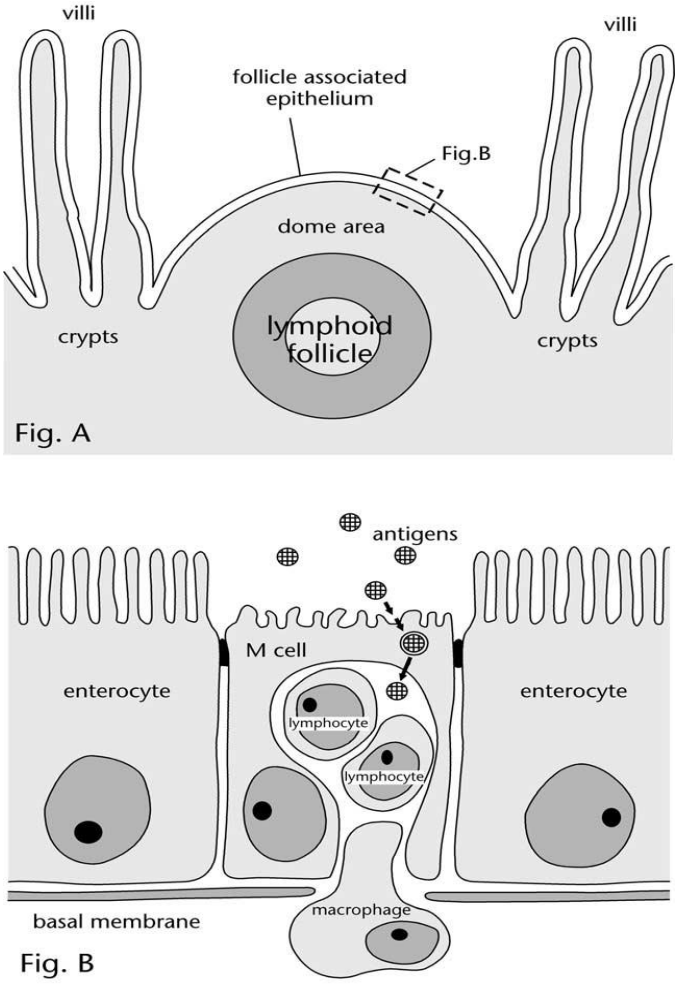



**Figure 2.5 A diagram illustrating the events leading to establishment of *Salmonella* infection of Peyer's patch in mouse (Jepson & Clark, 2001).**

## 2.7 Virulence factors

Establishment of *Salmonella* infection relies on virulence factors (molecules expressed and secreted by the pathogens) which enable them to colonize at a niche site in the host, evade the host's immune response, provide entry into and exit out of cells and obtain nutrition from the host. The ability of *Salmonella* to attach to the intestinal epithelium is attributed to a number of virulence factors that are related to the bacterial cell wall components such as fimbriae, flagella, endotoxins and outer membrane proteins.

### 2.7.1 Fimbria

A fimbria is a proteinaceous appendage found in *Salmonella* strains of which five types have been characterized (Thorns, 1995). Fimbriae are thought to enhance the ability of the bacteria to colonize and adhere to specific host target tissues in the early stages of infection. For example, serovars Gallinarum and Pullorum expressing *S. enterica* serovar Typhimurium type 1 fimbriae exhibited an increased ability of attachment and invasion efficiency to the human epithelial cells (Wilson, et al., 2000). Hence, fimbriae contribute to virulence. Darekar and Duguid (1972) found that the fimbriated *S.* Typhimurium caused more infections (+26%) and death (+40%) than the non-fimbriated strains in orally inoculated mice.

### 2.7.2 Flagella

Flagella are long, thread-like appendages which provide *Salmonella* with the ability to move, which increases the probability that they will reach suitable sites for invasion. In most serovars of *Salmonella,* flagella alternate between two distinct antigenic structures referred to as phase 1 and phase 2 encoded by the *fliC* and *fliB* genes respectively (Schmitt, et al., 2001). Flagella actively mediate the initial interaction between the bacterium and host cell and *Salmonella* mutants without flagella have reduced colonization capability in the gastrointestinal tract. Passive antibody activity directed against *Salmonella* flagella conferred protection from experimental salmonellosis in mice when administered orally using chicken egg yolk antibodies specific for *Salmonella* (Yokoyama, et al., 1998).

### 2.7.3 Toxin

*Salmonella* produce both exotoxins and endotoxins. The exotoxins can be further divided into two groups: cytotoxins and the enterotoxins. The major toxins of *Salmonella* causing intestinal and systemic infections are conferred by endotoxins that are an integral part of the

bacterial cell wall (van Asten & van Dijk, 2005). Gram-negative bacteria endotoxins are broadly represented by lipopolysaccharide (LPS) and elicit their toxic affect after attachment to cell-associated extracellular receptors. The O antigen component of LPS is not only immunogenic but also is virulent to the host by evasion of the innate host defences. During invasion of the gut, *Salmonella* organisms use LPS as a ligand mediating the internalization of bacteria by epithelial cells (Lyczak, et al., 2001).

### 2.7.4 Outer membrane proteins (OMPs)

In severe cases, *Salmonella* can spread from the gut, the site of infection, to cause a systemic disease. These pathogens must possess a number of virulent factors that help them to evade the host's defences, in addition to fimbriae, flagella and LPS. These virulent components are defined as outer membrane proteins (OMPs) which are most frequently associated with systemic infections (Valone & Chikami, 1991).

Porin is a trimer protein encoded by Omp C, Omp F and Omp D genes. Studies have revealed that proteins, especially porins, have been implicated in host defence mechanisms (Tabaraie, et al., 1994). Porins inhibit phagocytosis by activating the adenylate cyclase system (Di Donato, et al., 1986). They have also been found to induce a dose-related oedema following injection into the rat paw, due to release of a histamine from peritoneal cells (approx 5% mast cells) (Galdiero, et al., 1990). Specific immune responses directed against *Salmonella* derived OMP, extract and purified proteins have been shown to protect various animals from animals from salmonellosis (Li, et al., 2005; Meenakshi, et al., 1999; Ochoa-Reparaz, et al., 1996; Tabaraie, et al., 1994).

### 2.7.5 Other virulence factors

Establishment of *Salmonella* infection relies on virulence of the bacteria, which is encoded by *Salmonella*-specific virulence genes. These genes play important roles in both intraperitoneal and intravenous infections. Many effector proteins are encoded in pathogenicity islands (PI) which are large chromosomal DNA fragments contributing to virulence of *Salmonella* through changes in attachment, invasion, survival and replication within host cells (Ehrbar & Hardt, 2005; Hacker & Kaper, 2000).

Type III secretion system (T3SS) is composed of a protein appendage found in *Salmonella serovars* that acts as a "molecular syringe" to inject effector proteins directly into the host cell cytoplasm, where the proteins manipulate host cell signalling (Galan, 2001). So far

approximately 30 different genes have been characterized to comprise the T3SS. Priority has been given to two groups of genes in the *Salmonella* genome encoding for the T3SS; *Salmonella* Pathogenicity Island 1 (SPI-1) and *Salmonella* Pathogenicity Island 2 (SPI-2).

The *Salmonella* pathogenicity island 1 (SPI-1) gene is a major genomic locus which is responsible for intestinal penetration. It is acquired by plasmid or phage mediated horizontal gene fragment and is not found in *E. coli* or related organisms (Figure 2.4). Although SPI-1 contains invasion genes for the uptake of *Salmonella* into epithelial cells and the subsequent induction of intestinal epithelial cell signals that lead to an inflammatory response (Galan, 1996; Galyov, et al., 1997), it only contributes to the enteropathogenic stage of the infection, but is not required for systemic disease (Figure 2.7) (Galan, 1996; Wood, et al., 1998). In contrast to SPI-1, SPI-2 is essential for *Salmonella* survival and replication in systemic infections. Molecular analysis indicated that the effector proteins of SPI-2 T3SS are found in *S. Enterica* but not *S. Bongori*. Although the pathogenic mechanism is still under the investigation, some of effects of SPI-2 T3SS have been widely accepted. For example, it is known that SPI-2 confers on *Salmonella* the ability to survive and proliferate up to 10 fold within macrophages and host epithelial cells (Figure 2.7) (Cirillo, et al., 1998; Hensel, et al., 1998; Ochman, et al., 1996; Vazquez-Torres, et al., 2000). Interference with normal intracellular trafficking inhibits maturation of *Salmonella*-containing phagosomes to phago-lysosomes, measured in an *in vitro* assay. Moreover, SPI-2 is required to exclude nicotinamide adenine dinucleotide phosphate (NADPH) oxidase from *Salmonella*-containing vacuoles, allowing the microbe to escape oxidative death inside infected phagocytes (Vazquez-Torres, et al., 2000).

Some other genes such as *lpf* fimbrial operon also play a role in mediating bacterial adhesion. Fimbrial adhesion is encoded by *lpf* binding receptors which are expressed on the surface of host cells. The fimbrial adhesion can recognize and bind to the epitopes that are only present at apical membrane surface (Baumler, et al., 1996).

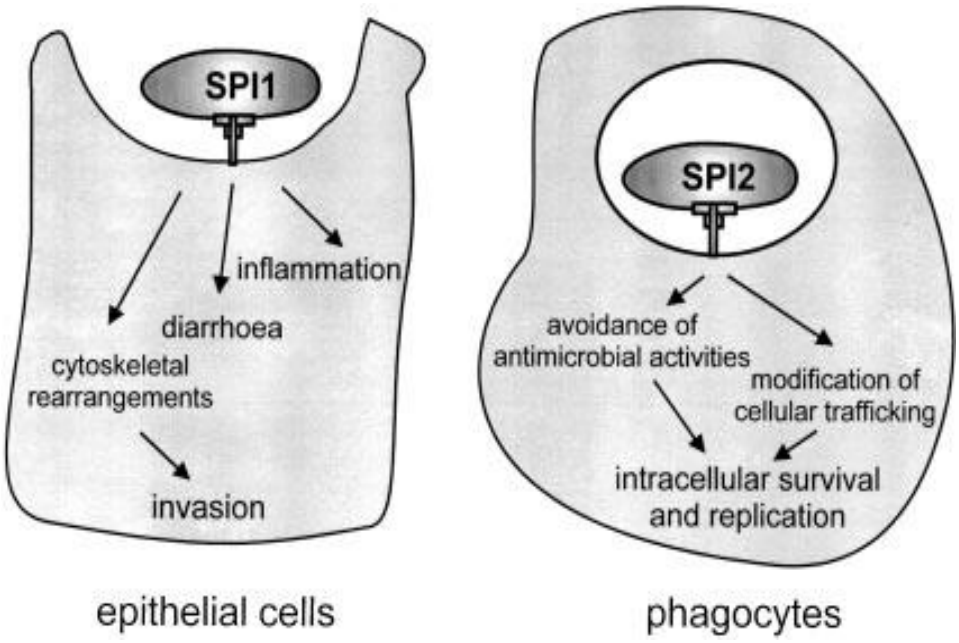



**Figure 2.6** ***Salmonella*** **enterica interaction with host cells. The function of the SPI-1-encoded T3SS is required for invasion of host cells and onset of diarrhoeal diseases. In contrast, the function of the SPI-2-encoded T3SS appears to be restricted to intracellular *Salmonella*. (Hensel, 2000).**

## 2.8 Host specificity of *Salmonella*

Currently, the species *Salmonella* enteric includes over 2500 closely related serovars. They are mainly associated with warm-blooded vertebrates and are responsible for most *Salmonella* infections in humans and domestic animals (Helm, et al., 2003; Uzzau, et al., 2000). *Salmonella* serovars differ in the range of host, such difference is referred to as serovar-host specificity (Uzzau, et al., 2001). Some *Salmonella* serovars are generalists, which mean they express wide ranging host infectivity, although their severity varies depending on the types of animal infected. For example, *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), usually causes a self-limiting gastroenteritis in human, while it can cause more serious systemic infections in hosts that are immunocompromised (Weinberger, et al., 2004). S. Brandenburg also infects many different host species (Clark, et al., 2004) but with varying pathology. Other *Salmonella* serovars are very restricted in their host range and display very narrow host infectivity and only cause systemic disease in one specific host. For instance, *Salmonella enterica* serovar Typhi (*S.* Typhi) is restricted to humans and causes systemic disease (typhoid fever). *Salmonella enterica* serovar Abortusovis (*S.* Abortusovis) is limited to infections in sheep (Edsall, et al., 1960; Pardon, et al., 1988).

Finally, some *Salmonella* serovars are host-adapted and express a differential capability of infecting particular hosts. They generally cause disease in one particular animal species, but may also cause disease in other animals under certain circumstances. For example *Salmonella enterica* serovar Dublin (*S.* Dublin) typically causes disease in cattle, but it can also infect humans, mice and sheep (Jack, 1971; Taylor, et al., 1982).

## 2.9 Immune response to *Salmonella* infection

The severity and outcome of a *Salmonella* infection depends on the virulent factors of antigens, the infectious dose, the genetic makeup and the host immunological status (Dunlap, et al., 1991; Richter-Dahlfors, et al., 1997) Both innate immunity (non-specific) and adaptive immunity (specific) play an important role to protect the host against *Salmonella*.

### 2.9.1 Innate immunity

The initial immune response to *Salmonella* infection is via the innate or non-specific immune system. It includes serum complement, and non-specific defence cells such as polymorphonuclear neutrophils, macrophages, and natural killer cells, which provide efficient 'front-line' defence against *Salmonella* invasion (Dietert, et al., 1991; Kogut, et al., 1994; Portnoy, 1992; Sharma & Schat, 1991). An important step to initiate the innate immune response against *Salmonella* is the recognition of the antigens on the bacterial surface, referred to as a pathogen–associated molecular pattern (PAMP). Upon infection, antigen-presenting cells (APCs) such as macrophages and DCs express a family of pattern-recognition receptors called Toll-like receptors (TLRs) on their surface. These receptors recognize conserved molecular products derived from various classes of pathogens, including Gram-positive and-negative bacteria, DNA and RNA viruses, fungi and protozoa (Werling & Jungi, 2003). Ligation between PAMP and TLRs initiates a signalling pathway that can stimulate the host defences through the induction of reactive oxygen and nitrogen intermediates (ROI and RNI) (De Groote, et al., 1996; Shiloh, et al., 1999; Shiloh, et al., 1997; Umezawa, et al., 1997).

Early bacterial growth is controlled in the reticuloendotelial system (RES) by the innate immune gene Natural-resistance-associated macrophage protein 1 (NRAMP1 also designated S1c11a11) with the contribution of both macrophages and polymorphonuclear cells (Conlan & North, 1992; Hormaeche, 1979a, 1979b, 1979c; Hu, et al., 1997; O'Brien, et al., 1979; Vidal, et al., 1993). Activated macrophages play a very critical role in antimicrobial immunity

and are regulated by NRAMP1. One of its pleiotropic effects on macrophage function is to regulate expression of major histocompatibility class II molecules (Lang, et al., 1997; Vazquez-Torres, et al., 2000). During this stage, phagocytic cells control the growth of invading *Salmonella* by either reactive oxygen intermediates (ROI) which are generated by NADPH oxidase (phox) or reactive nitrogen intermediates (RNI) produced by the inducible nitric oxide synthase (iNOS).

### 2.9.2 Adaptive immunity

Adaptive immune responses are often regarded as the second level of host defence and are initiated when T- and B- lymphocytes recognize foreign antigens presented on APCs (Cuadros, et al., 2004). Cell-mediated immunity and humoral immunity are two fundamental adaptive mechanisms conferred by T- and B- lymphocytes, which play an essential role in the clearance of *Salmonella*. Plasma cells produce antibodies which protect the host against infection by interacting with the surface of infecting pathogens to prevent them from bacterial attachment and invasion amongst other things (McGhee, et al., 1992; Michetti, et al., 1992). This protective function of antibodies occurs with the extracellular stage of bacterial infection. For cellular immunity, thymus derived lymphocytes (T cells) normally exert their effect on mediating the infection course by serving as direct effectors or function regulators. These cells are grouped according to their function in the immune system; cytotoxic (Tc) or helper (Th) T lymphocytes. The Th cell population can be further divided into Th1 and Th2 based on the different cytokines they elicit (Mosmann, et al., 1986). The Th1 cell is the producer of IFN-γ and IL-2, which are main components in cell-mediated immunity (CMI), while the Th2 cell produces interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin (IL-6) and interleukin-10 (IL-10) cytokines that affect the humoral immunity. Both T lymphocytes and cytokines are essential components for a protective immune.

#### 2.9.2.1 Cell-mediated immunity

In lethal *Salmonella* infections, bacteria rapidly reach large numbers in the tissues and death occurs within a short time by endotoxin poisoning. In sublethal infections, exponential growth of the organisms is prevented by host-response suppression in the RES towards the end of the first week, resulting in a plateau phase. The host response relies on chemo taxis to attract bone-marrow-derived macrophages (Hormaeche, et al., 1990; Mastroeni, et al., 1991; Mastroeni, et al., 1994; Mastroeni, et al., 1993; Nauciel & Espinasse-Maes, 1992). They contribute to the formation of granulomas rich in mononuclear cells.

Furthermore, several mediators such as tumor necrosis factor alpha (TNFα), interferon-gamma (IFN-γ), IL-12, and IL-18 contribute to this formation and are linked to the suppression of *Salmonella* growth in the tissue (Mastroeni, et al., 1999). Tumor necrosis factor alpha (TNFα) is constantly required for the control of virulent *Salmonellae* in the RES both in a sublethal primary infection in innately resistant mice and also in a secondary infection in innately susceptible mice immunized with a live vaccine, but may not be essential for bacterial clearance of avirulent organisms from the tissues (Galdiero, et al., 1993).

Interferon-gamma (IFN-γ) or type II interferon, with a molecular weight of 19,150 kDa in sheep, is crucial for the activation of macrophage and plays an important role in cell-mediated immunity (Young & Hardy, 1995). Although IFN-γ is produced predominantly by T lymphocytes and natural killer (NK) cells, other types of cells, such as B cells, natural killer T (NKT) cells and professional APCs are also involved in IFN-γ production (Carnaud, et al., 1999; Flaishon, et al., 2000; Frucht, et al., 2001; Gessani & Belardeli, 1998; Harris, et al., 2000; Yoshimoto, et al., 1998).

It has been revealed that IFN-γ expression is rapidly upregulated in the intestinal mucosa, Peyer's patches, mesenteric lymph nodes, spleen, liver and leukocytes in response to *Salmonella* infection in infected mice (Eckmann, et al., 1996; Ramarathinam, et al., 1991) and sheep (Li, et al., 2005). Neutralization of IFN-γ function with antibodies or 'knockout' IFN-γ gene leads to increases of bacterial number and decrease of host survival in mice (Bao, et al., 2000; Gulig, et al., 1997; Nauciel & Espinasse-Maes, 1992). On the contrary, elevated IFN-γ levels in the circulation can inhibit the bacterial growth and improve the number of mice that survive. Although IFN-γ can exert its effects in both phagocytic and non-phagocytic cells, the major way for IFN-γ to control *Salmonella* infection is by activating macrophages in order to kill *Salmonella* and improving the presentation to lymphocytes via the major histocompatibility complex (MHC). In addition, Kagaya et al. (1989) pointed out that activated macrophages can develop the ability to secrete reactive oxygen intermediates (ROI), as well as to kill non-specific facultative intracellular microorganisms and a variety of neoplastic cells when they are exposed to recombinant IFN-γ (rIFN-γ). The cytokines IL-12 and IL-18 play a protective role in host defence against invasion of *Salmonella* (Dybing, et al., 1999; Mastroeni, et al., 1999). This effect is likely to be regulated by IFN-γ, because the serum levels of IFN-γ are significantly lower after IL-12 and IL-18 neutralization and IL-12 and IL-18 secreted in macrophages have been shown to induce IFN-γ production. It was suggested that macrophages are activated through producing IL-12 p40 which functions as a signal to IFN-γ. Neutralization of IL-12 blocks the pathway between macrophage and IFN-γ,

resulting in exponential growth of bacteria and a decrease in host survival whereas elevating the serum level of IL-12 increases host survival (Kincy-Cain, et al., 1996).

Apart from direct anti-microbial activities, IFN-γ also takes part in the stimulation of antibody production. It induces the increase of IgG2 and lowers the IgG1 levels in cattle (Estes, et al., 1994). IFN-γ also stimulates T cell proliferation and development (Doucet & Bernard, 1997; Theze, 1999).

Lymphocyte proliferation is a key indicator of the host defence response to invasion of pathogens. It is likely that proliferation of T cells *in vitro* parallels protective immunity *in vivo*. Li et al. (2005) found that the administration of a semi-purified porin-rich vaccine induced a proliferative response to the same antigen or LPS *in vitro*, in sheep. Conversely, Doucet and Bernard (1997) found that infection with *Salmonella* Abortusovis in sheep could suppress the lymphoproliferative response *in vitro* to Concanavalin A (Con A), a non specific mitogen. It has been shown that the CD4+ T cell percentages decreased whereas B and MHC-II+ cell percentages increased in infected sheep (Doucet & Bernard, 1997).

#### 2.9.2.2 Humoral immunity

Although *Salmonella* infections are primarily controlled by cellular immunity, the absence of humoral immunity leads to inefficiency in *Salmonella* clearance as antibodies function by binding to the invading microbe and neutralizing it or facilitating uptake opsonisation by macrophages and its elimination (Moore, et al., 2003; Pier, et al., 2004). It has been demonstrated that antibodies involved in protection against *Salmonella* infection include immunoglobulin M (IgM), IgG and IgA (Husband, 1978; Iankov, et al., 2002; Mukkur, et al., 1995). By the first week after infection, antibody response can be detected in the sera of different species such as chicken (Lee, et al., 1981), sheep (Brennan, et al., 1994), and cattle (Lindberg & Robertsson, 1983). IgM is the first isotype elicted among the different isotypes of anti-*Salmonella* antibodies, followed by IgG and IgA (Hassan, et al., 1991).

Immunoglobulin M (IgM), which accounts for 5-10% of the total serum immunoglobulin, is structurally a pentamer. *In vivo*, IgM was ten times more effective than IgG in promoting removal of *S.* Typhimurium from blood after intravenous infection and a thousand times more effective than IgG in promoting killing of the bacteria after intraperitoneal challenge (Saxen, 1984). Furthermore IgM can amplify the immune response by functions as a powerful activator of the complement system.

Immunoglobulin G (IgG) is the most abundant class in serum and constitutes approximately 80% of the total serum immunoglobulin. It protects the body against *Salmonella* by agglutination and immobilization, complement activation (classical pathway), opsonisation for phagocytosis and neutralization of their toxin (Paul, 1993). It has been shown that serum levels of antibody IgG were linked to host protection against infection with *Salmonella* in many species such as mice, sheep (Bernard, et al., 2002), chickens (Iotova, 1982) and cattle (Yokoyama, et al., 1998).

Immunoglobulin A (IgA) is produced by many species. IgA antibody which constitutes only a small proportion (10-15%) of the total immunoglobulin in serum is more predominant in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary and digestive tracts. Secretory IgA (S-IgA), constitutes > 80% of all antibodies produced in the mucosa-associated tissues of human and mice, inhibits bacterial adherence, neutralizes toxin moieties, mediates protection against infection through antibody-dependent cellular cytotoxicity, initiates bactericidal action by iron-sequestering compounds and serves as a possible opsonin for mucosal phagocytes (Funakoshi, et al., 1982; Kilian, et al., 1988; Tagliabue, et al., 1983). For example, Michetti et al. (1992) demonstrated that the monoclonal S-IgA, directed against a surface epitope of *S.* Typhimurium, could prevent systemic disease caused by invasive enteric pathogen.

## 2.10 Validity of *in vitro* culture methods

Abortion due to *S.* Brandenburg is one of the most important problems in ovine reproduction and breeding in regions of New Zealand (Boxall, et al., 1999). The pathogenesis of abortion events are difficult to study *in vivo*, because of surgical inaccessibility and associated high experimental costs. Cell culture provides a low cost model for studying the mechanism of abortion caused by *S.* Brandenburg *in vitro*. Cells can be infected with *S.* Brandenburg and the infective characteristics of the bacteria in various cell lines can be compared. Similar methods have been used to investigate the virulence and host specificities of other *Salmonella* serovars such as Dublin (Pullinger, et al., 2007), Gallinarum (Paulin, et al., 2002) and Choleraesuis (Paulin, et al., 2007) in different hosts including cattle, chicken and pigs. In preliminary experiments with *S.* Brandenburg, there appears to be good correlation between invasion characteristics *in vitro* (primary sheep in intestinal cells or immortalised human cell lines) and *in vivo* (ovine ligated ileal loops) experiments (Brandt, et al., 2008).

In summary, infections due to *S.* Brandenburg remain a serious problem for New Zealand sheep industry, although partial protection can be provided by changes in farm management

and vaccination. Understanding the infection characteristics (adhesion, invasion and replication) of *S.* Brandenburg in sheep may lead to improved methods of disease control.

# Chapter 3
# Materials and Methods

All experimental procedures concerning the handling of pathogens were approved by the Lincoln University Institutional Bio-safety Committee.

## 3.1 Sample collection and experimental design

Epithelial cells were isolated from the oviduct and ileum of recently slaughtered two-year-old (two-tooth) non-pregnant ewes or two-year-old non-pregnant cows. A variety of methods were compared in order to optimise cell yield and viability. Adhesion to, invasion of, and replication within, *ex vivo* and *in vitro* cultured oviduct epithelial cells (OECs) and intestinal epthelial cells (IECs) by *S.* Brandenburg and *S.* Typhimurium isolates, were quantified by means of infection assays (Watson, 1995). This assay was designed as 4 grouped experiments based on the hypotheses outlined on p14.

In experiment 1, different profile 14 isolates of *S.* Brandenburg derived from sheep foetus, cattle foetus and human blood (3684, 3062 and 4468, respectively) or a non-profile 14 isolate of *S.* Brandenburg from pet food (4527), were added into 24 h or 7 day old cell cultures derived from sheep oviduct. Three independent assays measured adhesion, invasion and replication at 1, 2, and 24 h respectively (Table 3.1).

In experiment 2, 24 h-old or 7 day-old cell cultures from sheep oviduct were infected with either the epidemic *S.* Brandenburg isolate (3684) or *S.* Typhimurium isolate (1979), adhesion, invasion and replication were compared (Table 3.1).

In experiment 3, *S.* Brandenburg isolate (3684) was added to 24 h-old or 7 day-old cell cultures either from bovine or ovine oviduct and adhesion, invasion and replication were compared (Table 3.1).

In experiment 4, *S.* Brandenburg isolate (3684) was added to 7 day-old cell cultures derived from either ovine small intestine or ovine oviduct and bacterial adhesion, invasion and replication were compared (Table 3.1).

**Table 3.1 Experimental design.**

<table>
<tr><th></th><th colspan="2">Ex vivo (24 h-old cell culture)</th><th colspan="2">In vitro (7 day-old cell culture)</th></tr>
<tr><td rowspan="4">Experiment 1<br>(repeat × 3)</td><td rowspan="4">OOEC</td><td>3684</td><td rowspan="4">OOEC</td><td>3684</td></tr>
<tr><td>3062</td><td>3062</td></tr>
<tr><td>4468</td><td>4468</td></tr>
<tr><td>4527</td><td>4527</td></tr>
<tr><td rowspan="2">Experiment 2<br>(repeat × 3)</td><td rowspan="2">OOEC</td><td>3684</td><td rowspan="2">OOEC</td><td>3684</td></tr>
<tr><td>1979</td><td>1979</td></tr>
<tr><td rowspan="2">Experiment 3<br>(repeat × 3)</td><td>OOEC</td><td rowspan="2">3684</td><td>OOEC</td><td rowspan="2">3684</td></tr>
<tr><td>BOEC</td><td>BOEC</td></tr>
<tr><td rowspan="3">Experiment 4<br>(repeat × 3)</td><td colspan="4">In vitro (7 day-old cell culture)</td></tr>
<tr><td colspan="2">OIEC</td><td rowspan="2">3684</td></tr>
<tr><td colspan="2">OOEC</td></tr>
</table>

**OOEC**: Ovine Oviduct Epithelial Cell.

**BOEC**: Bovine Oviduct Epithelial Cell.

**OIEC**: Ovine Intestinal Epithelial Cell.

**3684**[1]: *S.* Brandenburg isolated from sheep.

**3062**[1]: *S.* Brandenburg isolated from cattle.

**4468**[1]: *S.* Brandenburg isolated from human.

**4527**[2]: *S.* Brandenburg isolated from pet food.

**1979**: *S.* Typhimurium isolated from sheep.

[1] PFGE genotype profile 14.

[2] PFGE genotype non-profile 14.

## 3.2 Oviduct epithelium primary culture from sheep and cattle

A primary cell culture was established by adding the cells lining the oviduct or gastrointestinal tract to growth medium. This involved 3 stages: (1) acquisition of the tissue sample and isolation of the cells; (2) mechanical or enzymatical disaggregation of the cells; (3) culture after seeding into the tissue culture flasks or plates (Freshney, 2005). All equipment and solutions used in the subsequent procedure were sterile.

### 3.2.1 Isolation of epithelial cells from ovine or bovine oviduct

Ewe or cow uteri, obtained from a local abattoir, were transported on ice to the laboratory. Once in the laboratory, the oviducts were excised from the reproductive tract, sterilized externally with 70% ethanol, and the excess connective tissue was trimmed off. After trimming, the cells from the inner lining of the oviduct were dislodged into the lumen of the oviduct by lightly pressing a clean microscope slide over the exterior surface of the oviduct, directed from the isthmus to the ampulla (Figure 3.1). A blunt 18 g needle was inserted into the utero-tubal junction (UTJ) and the epithelial cells were flushed out with about 2-5 ml Dulbecco PBS (GIBCO) at 37$^{o}$C and added to 10 ml 37$^{o}$C Dulbecco PBS in a 50 ml centrifuge tube (Way, 2006). The tube was inverted to suspend cells and left for 10 min to allow the cells to settle to the bottom of the tube, and the supernatant was aspirated. If many cells remained suspended in Dulbecco PBS, the above process was repeated two to three times, and the resultant cells were resuspended gently in 8 ml D-MEM (GIBCO). All the procedures were carried out at room temperature.

### 3.2.2 Disaggregation of epithelial cells from ovine or bovine oviduct

For the 24 h cultures, newly isolated epithelial cell clumps in 8 ml D-EMEM were directly placed in a 25 cm$^2$ NUNC tissue culture flask. To the cells was added 2 ml of 2000 units/ml collagenase (Sigma) and 100 µl of 0.06% (w/v) DNase I (Sigma) followed by incubation for 3-5 h at 37$^{o}$C, with 5% $CO_2$. The cells were then centrifuged at 800 rpm for 10 min and the supernatant was discarded. The cell pellet was resuspended in Dulbecco PBS and re-centrifuged in order to wash off the collagenase and DNase I in the medium.

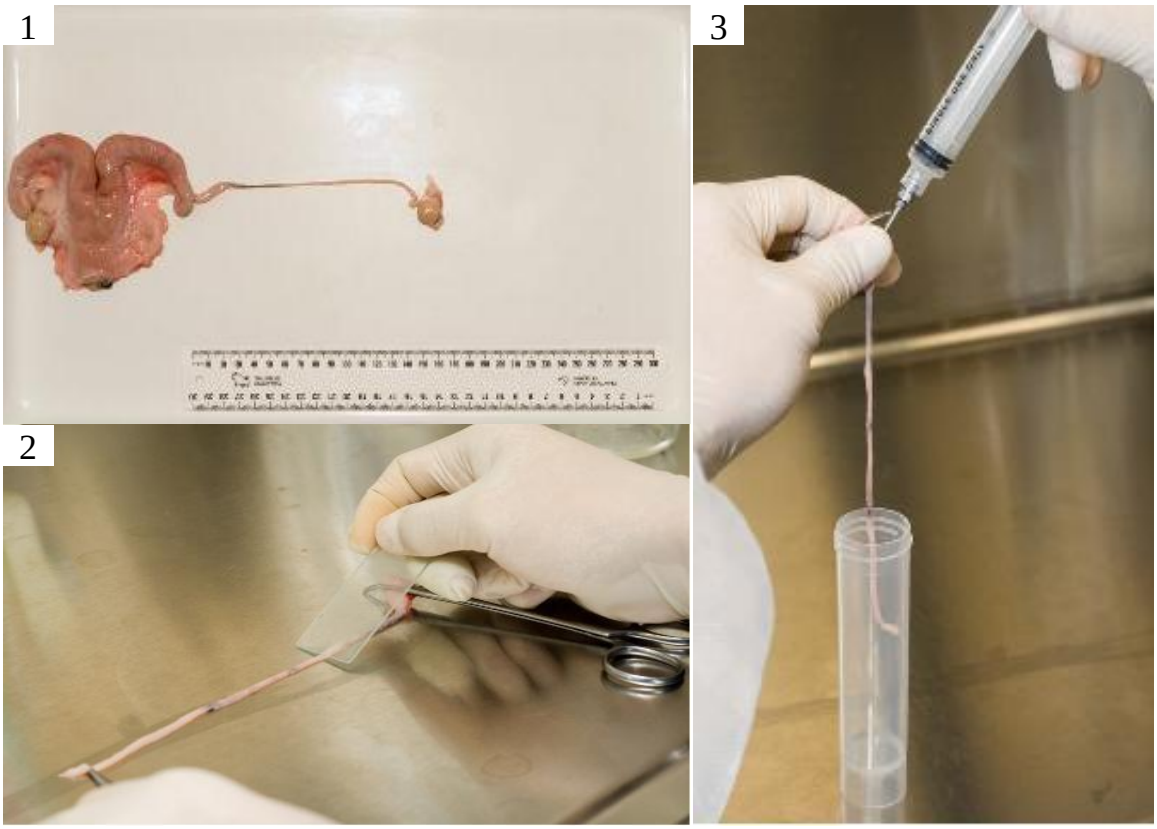



**Figure 3.1** **(1) Rinsed and trimmed ovine oviduct. (2) Microscope slide pulls along the exterior surface of the oviduct toward the infundibulum (right). (3) Epithelial cells are flushed into warm, sterile Dulbecco PBS solution.**

The cell wash–centrifugation step was repeated twice and the cell pellet then was suspended in 2.5 ml of trypsin-EDTA that contained 2.5 mg/ml trypsin and 0.38 mg/ml Na EDTA (GIBCO), followed by 20-30 min of incubation. After disaggregation with the above combination of collagenase, DNase I and trypsin-EDTA, most cells were individual. Any small undigested cell clumps were disaggregated mechanically using a 1 ml pipette. The disaggregated oviduct epithelial cells (OECs) in trypsin-EDTA were diluted with D-MEM complete growth medium (supplemented with 10% or 20% FBS). A variety of other combinations to effect disaggregation (enzyme type, concentration, incubation time and mechanical) were also trialled before this procedure was decided on (Table 3.2). For the 7 day-old cell cultures, newly isolated cell clumps were immediately transferred into complete growth medium and cultured for 7 days at 37$^{o}$C with 5% $CO_2$. After 7 days, the cell monolayers were washed with Dulbecco PBS and detached by the addition of 2.5 ml of typsin-EDTA for 20-30 min at 37$^{o}$C incubated with 5% $CO_2$. The detached cells were then diluted in D-MEM containing 10% or 20% FBS and the viability was assessed after adding 0.4% (w/v) trypan blue and viewed by light microscopy.

**Table 3.2 Enzyme combinations tested for disaggregation of OECs.**

| Enzymes | | | Time | |
|---|---|---|---|---|
| **DNase I (0.6 mg/ml)** | **Collagenase (400 units/ml)** | **Trypsin-EDTA (2.5 mg/ml)** | **Incubation1 ($37^oC$)** | **Incubation2 ($37^oC$)** |
| ✓ | | | 3 h | |
| | ✓ | | 3 h | |
| | | ✓ | 30 min | |
| ✓ | | ✓ | 3 h | 30 min |
| | ✓ | ✓ | 3 h | 30 min |
| ✓ | | | 3 h | |
| ✓ | | ✓ | 3 h | 30 min |

### 3.2.3 Seeding the oviductal cells onto the culture plates

For the 24 h-old cell cultures, the disaggregated cells were seeded in 24-well poly-l-lysine (Sigma) pre-coated culture plates. The recommended working concentration of poly-l-lysine was 0.1 mg/ml and 400 µl was loaded into each well. A gentle swirl was given to the plate to ensure coverage. The plates with poly-l-lysine were placed in incubation at $37^oC$ with 5% $CO_2$ for 3 h. The excess reagent was removed and wells were rinsed twice with sterile water; followed by Dulbecco PBS, and then D-MEM containing 10% FBS.

Then, 1 ml cell culture medium containing 5.0-5.2 $\log_{10}$ OECs were placed in each of the coated wells and incubated for 20-22 h at $37^oC$ with 5% $CO_2$ and maximum humidity.

For the 7 day-old cell cultures, 1 ml of trypsin-EDTA disaggregated cells at 5.0-5.2 $\log_{10}$ per ml were sown in 24-well culture plates (without coating with poly-l-lysine) and cultured for 12-15 h at $37^oC$ with 5% $CO_2$ and maximum humidity.

## 3.3 Small intestinal epithelium primary culture from sheep

### 3.3.1 Isolation of epithelial cells from ovine small intestine (ileum)

The method to isolate intestinal epithelial cells (IECs) is derived from that of Evans et al. (1992). Epithelial cells were isolated from the distal ileum of slaughtered sheep collected at a local abattoir. Approximately 15-20 cm of ileum was placed in RPMI 1640 containing 10% (v/v) FBS, 100 µg of streptomycin/ml, 100 U of penicillin/ml, and 2.5 µg of amphotericin

B/ml (GIBCO) and then transported on ice to the laboratory. The ileum was then cut into 2 cm × 2 cm sections. The sections were placed in Hanks balanced salt solution (Bio-Whittaker) without $Ca^{2+}$ or $Mg^{2+}$ containing 0.1 mM EDTA (BDH) and 0.1 mM dithiothreitol (BDH) and incubated at 37°C for 10 min with vigorous shaking (Figure 3.2). The supernatant was then removed and the tissue was chopped finely and placed in RPMI 1640 containing 0.05% (w/v) collagenase (Sigma) and incubated at 37°C for 15-20 min with vigorous shaking. After this procedure, the supernatant consisting of intestinal crypts and single cells was removed with a Pasteur pipette, pelleted by centrifugation at 1000 × *g* for 5 min, and resuspended in D-MEM medium containing 10% (v/v) FBS and kept on ice. Fresh collagenase solution was added to the biopsy tissue repeatedly two to three times until no more cells could be isolated. The pellet was gently resuspended in 10 ml D-MEM plus 10% (v/v) FBS, 0.5 ng/ml of epidermal growth factor (EGF) (Sigma), 8 µg/ml of insulin (Sigma), 10 µg/ml of gentamycin (GIBCO), 50 µg/ml of hydrocortisone (Sigma), 100 µg/ml of streptomycin, 100 U/ml of penicillin (GIBCO), and 2.5 µg/ml of amphotericin B (GIBCO) (Hashim, et al., 2004).

### 3.3.2 Seeding the IECs onto the culture plates

The IEC attachment rates can be improved by coating the surface with the attachment factor collagen Type I (BD). Collagen Type I was diluted to 300 µg/ml in Dulbecco PBS and 650 µl of the working collagen solution was added into each well of the 24-well plates, followed by incubation at 37°C with 5% $CO_2$ and maximum humidity for 3 h. After 3 h of incubation, the plate covers were removed and the plates were placed in a sterile hood overnight to dry the collagen at room temperature. The wells were washed twice with sterile water, and the washing was repeated using Dulbecco PBS and D-MEM. Plates were air dried overnight and stored at 4°C.

One ml of the D-MEM containing approximately 5.0-5.2 IECs /ml were added onto the collagen-coated plate and cultured for 7 days, at 37$^{o}$C with 5% $CO_2$ and maximum humidity.

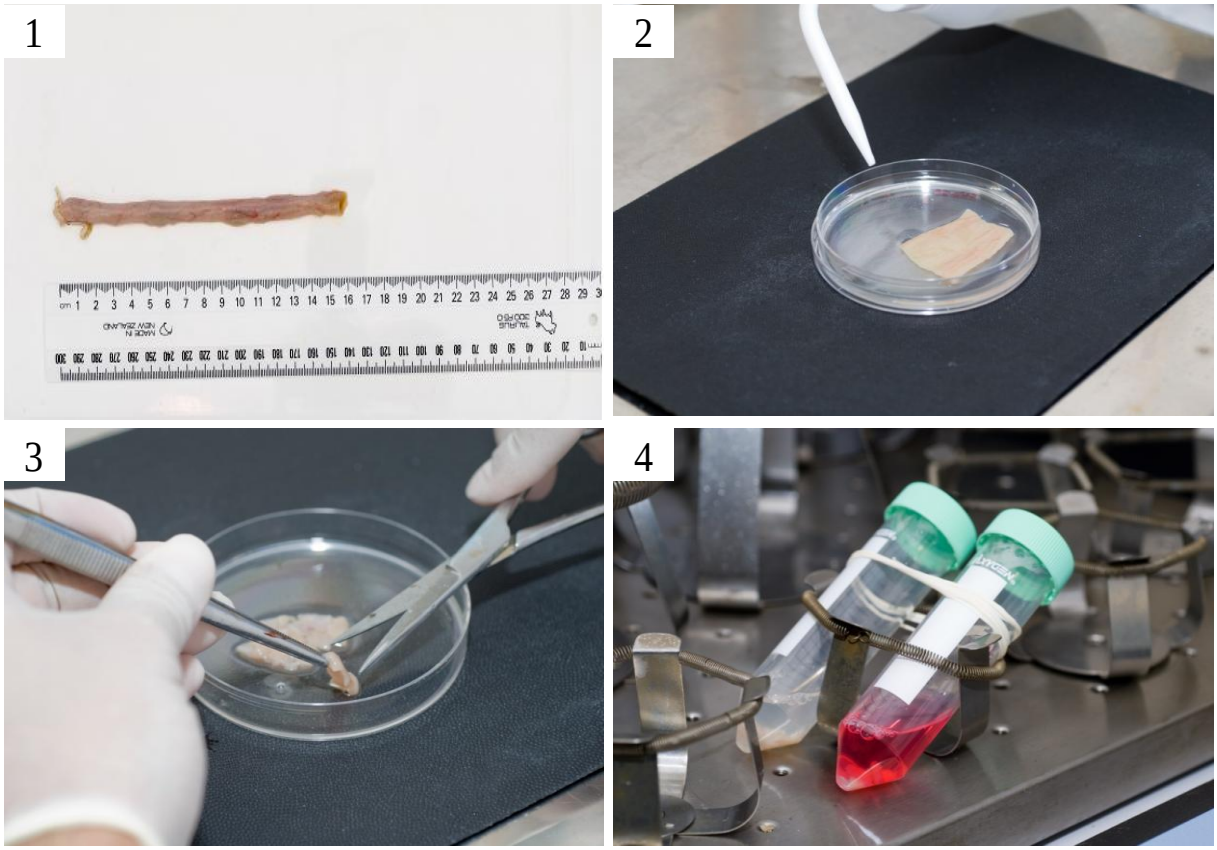



**Figure 3.2** **(1) Section of distal ileum (15-20 cm). (2) Washing of ileum (2 × 2 $cm^2$). (3) Fine dicing of ileum. (4) Incubation with 0.1 mM EDTA and DTT in RPMI 1640 medium with 0.05% collagenase.**

## 3.4 Characterisation of oviduct and small intestinal epithelial cells

### 3.4.1 Cell viability and yield examination of cultured cells

The morphology of newly isolated epithelial cells from sheep and cattle oviduct and ileum were examined by light microscopy after 1 h, 24 h or 7 days of culture. The viability and number of disaggregated cells were evaluated on a haemocytometer, after staining in trypan blue (GIBCO); 20 µl of cell homogenate was diluted in the equal volume of 0.4% (w/v) trypan blue and then counted.

### 3.4.2 Haematoxylin and Eosin (H&E) staining of cultured OECs

Staining with haematoxylin and eosin (H&E) was carried out as per Walter (1995). The OECs that had been cultured for 24 h or 7 days on poly-l-lysine pre-coated cover slips, were fixed using 4% formaldehyde for 30 min. Cells were then stained with 2-5 ml of Harris haematoxylin (BDH) and incubated for 30 min at room temperature. After 30 min incubation, the haematoxylin solution was washed off with sterile water and cells were incubated in 2-5

ml of eosin for another 30 min at room temperature. Cells then were rinsed with Dulbecco PBS, mounted in 100% glycerol and examined by light microscopy.

### 3.4.3 Giemsa staining of cultured IECs

Giemsa staining is specific for the phosphate groups of DNA and attaches itself to regions of DNA where there are high amounts of adenine-thymine bonding. IECs that had been cultured for 7 days on collagen Type I pre-coated cover slips were fixed with 4% formaldehyde for 30 min. The cell monolayer was incubated at room temperature in freshly prepared Giemsa (Sigma) stain for 1 h. The cell monolayer was then rinsed in 0.5% (w/v) aqueous acetic acid for 30 min, followed by Dulbecco PBS, mounted in 100% glycerol and examined by light microscopy.

### 3.4.4 Immunohistochemical characterization of cultured cells

Indirect immunohistochemical staining of cytokeratin was performed as per Tiemann et al. (2001) using a monoclonal antibody to cytokeratin 8 and 18 (Sigma), that is, specific for epithelial cells. The cells were fixed with 4% formaldehyde in Dulbecco PBS for 30 min, permeabilized with 1% (v/v) Triton X-100 in Dulbecco PBS for 30 min, blocked with 0.5% (w/v) bovine serum albumin (BSA) for another 30 min and then incubated with the anti-PAN cytokeratin 8 and 18 primary antibody at 10 µg/ml in 0.5% (w/v) BSA, for 12 h at 4$^{o}$C. The monolayer was rinsed with Dulbecco PBS 3 times, incubated with 10 µg/ml sheep anti-mouse IgG secondary antibody coupled to FITC (Sigma) in 0.5% (w/v) BSA for another 12 h at 4$^{o}$C, mounted in 100% glycerol and fluorescence was viewed by microscopy (Leica) at 400 nm.

## 3.5 Optimisation of infection assays

### 3.5.1 Growth characteristics of *Salmonella* cultures

In order to assess the growth characteristics of the *Salmonella* isolates in Luria-Bertani (LB) broth and determine the exponential growth phase, 1.4 ml of pre-cultured (12-15 h at 25$^{o}$C) bacteria (isolates 3062, 3684, 4468, 4527 and 1979) was added into 36 ml of Luria-Bertani (LB) broth. The LB broth containing bacteria was then incubated in a 37$^{o}$C water bath. Culture absorbance was measured ($OD_{600}$) at 0, 2, 4, 6, 8, 10 or 12 h. As each time point, a mean of 3 absorbances was calculated and growth curves were generated.

### 3.5.2 Efficacy of gentamycin

To determine the ability of gentamycin to inactivate extracellular *Salmonella* isolates, 100 μg/ml of gentamycin (GIBCO) was added to D-MEM nutrient medium without antibiotics. Overnight cultures of bacteria were added to the medium and incubated for 2 h at 37°C in 5% $CO_2$. Samples of 200 μl were taken immediately after the addition of gentamycin and at 30 min intervals and culture on LB agar plates that were incubated at 37°C overnight and *Salmonella* colony-forming units (CFUs) were quantified.

### 3.5.3 Lytic buffer viability assay

An assay was performed to determine whether *Salmonella* could survive exposure to sodium deoxycholate. *Salmonella* isolates were grown overnight at 37$^{o}$C in broth culture and 200 μl aliquots were taken and centrifuged at 10,000 rpm for 10 min. The bacterial pellet was then resuspended in 200 μl of lytic buffer (0.1% sodium deoxycholoate in Dulbecco's PBS). Samples in triplicate were taken after 10 and 30 min and independently counted with a 10-fold serial dilution. Bacterial viability was determined on LB agar after overnight incubation at 37°C.

## 3.6 *Salmonella* infection assays

### 3.6.1 Epithelial cell culture preparation

The culture methods followed as per Galan and Curtiss (1989). Cells were inoculated in a 24 well plate at approximately 5.0-5.2 $\log_{10}$ cells per ml in D-MEM containing 10% (v/v) FBS and 1% penicillin/streptomycin (Watson et al., 1995). The plates were incubated for 24 h at 37$^{o}$C after which the monolayer was washed once with unsupplemented media and 1 h later the bacteria were added.

### 3.6.2 Bacterial strains and growth conditions

*S.* Brandenburg strains isolated during 1996-2006 from sheep, cattle, human and pet food and a *S.* Typhimurium strain isolated from sheep in 2010 were obtained from the ESR Enteric Reference Laboratory (Table 3.3).

**Table 3.3 Bacteria strains used in adhesion, invasion and replication assays.**

| Strain | NZRM Accession No. | Source | Host Species |
|---|---|---|---|
| *S.* Brandenburg | 3062 | Foetus | Cattle |
| | 3684 | Foetus | Sheep |
| | 4468 | Foetus/Blood | Human |
| | 4527 | Pet food | Cattle |
| *S.* Typhimurium | 1979 | Faeces | Sheep |

For each assay, a vial of bacterial cells was thawed and cultured on LB agar plates (Sigma) overnight at 37°C. For liquid culture, a scraping of a colony was taken from the culture plate and transferred to 5 ml of LB broth (Sigma), followed by overnight incubation at 25$^{o}$C with vigorous shaking at 150 rpm (Figure 3.3).

For inoculum preparation, the OD (optical density) of the *Salmonella* cultures in LB broth was measured for adequacy and 200 µl was diluted in 5 ml of fresh LB and incubated with shaking at 37$^{o}$C for a further 2 h. The resultant OD was expected to be between 0.6 and 0.8, the *Salmonella* culture was diluted down to an OD of 0.3 and 15 to 30 µl of the *Salmonella* culture was added to 15 ml unsupplemented, pre-warmed D-MEM to give a final bacterial concentration of 6.0-6.5 $\log_{10}$ CFUs/ml.

### 3.6.3 Enumeration of bacteria

Bacterial counts were performed after 10-fold serial dilutions by plating out culture onto LB agar and assessing after overnight incubation at 37$^{o}$C. The number of bacteria were calculated using a modification of the method by Miles et al. (1938). Each serial dilution was assessed and a count was undertaken when the number of organisms on the agar plate ranged from 10 to 100 CFUs. *Salmonella* recovered in D-MEM without cells were termed ‘media control’, those recovered before the addition of gentamycin were termed ‘adhesion’, those recovered after the gentamycin treatment ‘invasion’, and those recovered after a further 24 h incubation with the gentamycin treatment ‘replication’ (Figure 3.3).

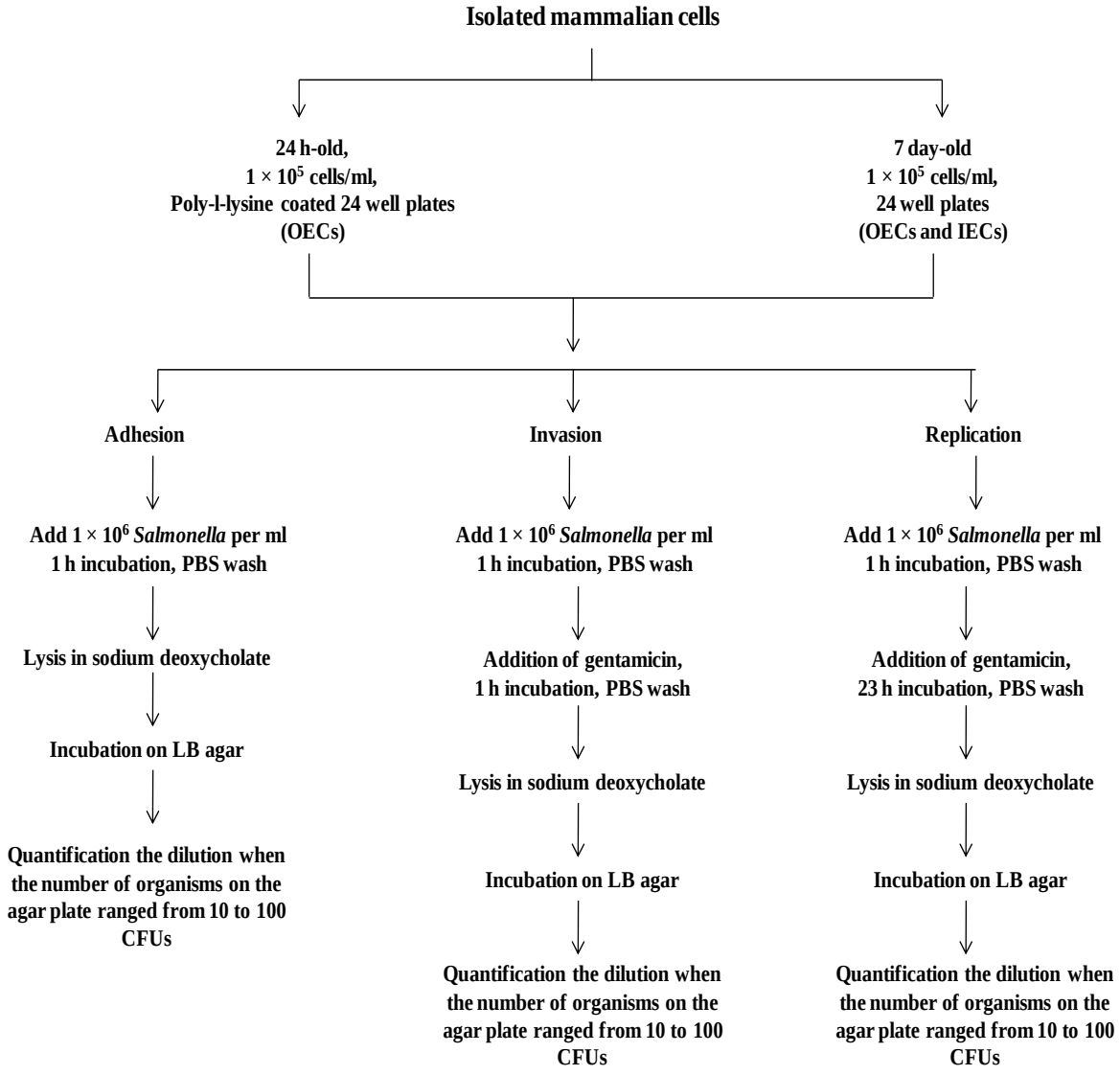



**Figure 3.3 Flow chart of *Salmonella* infection assays.**

### 3.6.4 Media control

The ability of bacteria to replicate over 1 h was measured by adding *Salmonella* inoculum into the wells without cells. This equated to the number of bacteria 'available' for adhesion. The bacteria in the D-MEM were counted as CFUs on LB agar after incubation at 37$^{o}$C for 12-15 h.

### 3.6.5 Adhesion assay

Oviduct epithelial cells having been cultured for 24 h (*ex vivo*) or 7 day (*in vivo*) were washed with 1 ml Dulbecco PBS to remove antibiotics before infection with bacteria. In triple wells, cells were infected with 1 ml of *Salmonella* inoculum, which equated to 6.0-6.5 $\log_{10}$ CFUs/ml bacteria, giving a multiplicity of infection (MOI) of 1:15-20, incubated at 37$^{o}$C for 1

h, and washed to remove the un-adhered bacteria (Figure 3.3). Thereafter, 1 ml of lytic buffer in Dulbecco PBS was added to each well, and aspirated 5 times. Bacteria in the lytic buffer were quantified on LB agar plates after incubation at 37°C for 12-15 h.

### 3.6.6 Invasion assay

The invasion assay was performed separately; one ml of *Salmonella* inoculum was added to each well of a 24-well plate, in triplicate, for each isolate. After 1 h of incubation, the monolayers were washed once with Dulbecco PBS and incubated for a further 1 h in un-supplemented medium containing 100 µg/ml gentamycin (Figure 3.3). This method differentiates bacteria that can invade the host cells and those which do not invade. Gentamycin added to the cultures does not penetrate eukarytic cells and therefore can not kill the bacteria that are already internalized. The *Salmonella* associated with the monolayers after the 1 h incubation with gentamycin were recovered by lysis of the cultured cells with 1 ml of Dulbecco PBS containing lytic buffer. Bacteria in the lytic buffer were quantified on LB agar plates after incubation at 37°C for 12-15 h.

### 3.6.7 Replication assay

The replication assay was carried out separately to test the replicating abilities of *Salmonella*. One ml of *Salmonella* inoculum was added to each well of a 24-well plate, in triplicate, for each isolate. After 1 h of incubation, the monolayers were washed once with Dulbecco PBS and incubated for a further 23 h in un-supplemented D-MEM containing 100 µg/ml of gentamycin (Figure 3.3). One ml of 0.1% sodium deoxycholate in Dulbecco PBS was then added to each well to release the intracellular bacteria. Bacteria in the sodium deoxycholate were quantified on LB agar plates after incubation at 37°C for 12-15 h.

## 3.7 Statistical analysis

The adhesive, invasive and replicative characteristics of the different *Salmonella* isolates when mixed with the different cell types were assessed statistically. Three samples of each dilution were tested, and counted in triplicate. Each adhesion, invasion and replication assay was independently carried out in 3 separate preparations of OECs/IECs. The viable-count data were analysed using parametric analysis (Student two-sample *t*-test) to assess mean differences between source of cells (species and organ) and *Salmonella* isolate.

The variances of the observations in each group were not equal and the two sample sizes were not equal hence the raw data was logarithmically transformed for normalization. GenStat ($12^{th}$ Edition, UK) and Sigmaplot (11.0, USA) was used for statistical analysis and plotting of data, respectively. *P*-values of difference between two groups of observations were obtained by referring the calculated value of the test statistic to the t-distribution table. For the adhesion, invasion and replication assays a $P<0.01$ was regarded as significant.

# Chapter 4

# Results

## 4.1 Oviduct epithelial cell (OEC) cultures

### 4.1.1 Cell morphology, yield and viability

Following isolation, observation by light microscopy showed that bovine and ovine epithelial cells were contained in aggregates with vigorously beating cilia on any remaining aggregate surfaces (Figure 4.1).

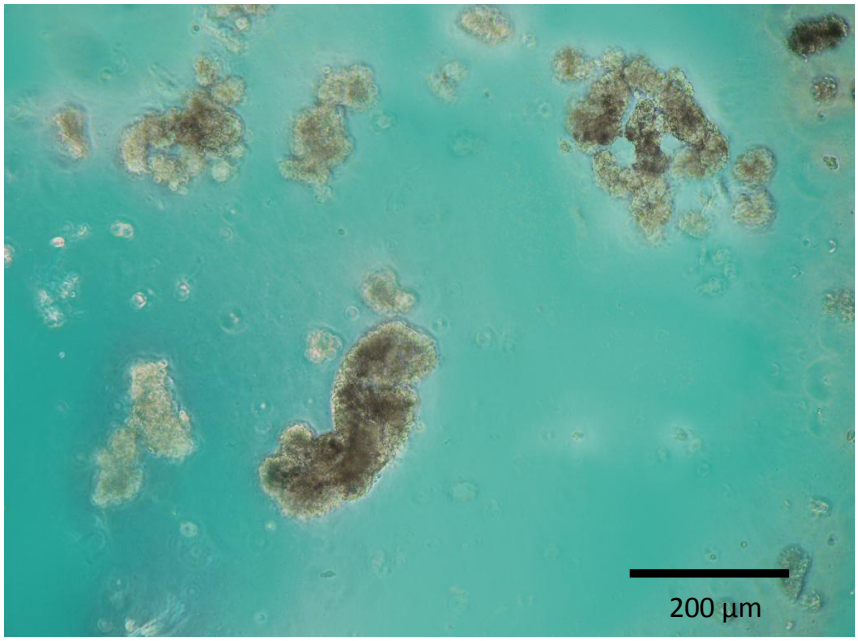



**Figure 4.1 Aggregates of OOECs in Dulbecco PBS following isolation.**

The yield of isolated OECs from sheep and cattle ranged from 7.4-7.74 $\log_{10}$ cells and 7.65-7.90 $\log_{10}$ cells per oviduct respectively. In both species, trypan blue staining was positive in the majority of singular cells and those cells on the periphery of the cell clumps, while those cells on the inside of the cell clumps, which made up the vast majority of the total cells, were all viable at 1 h after isolation (Figure 4.2a).

In order to disaggregate the OECs for the *ex vivo* assay, newly isolated epithelial cells were treated with a combination of collagenase, DNase I and Trypisn-EDTA for 3 h. Several kinds of enzymatic combinations were trialled before the optimal procedure was decided on (Table 4.1). As indicated in Table 4.1, trypsin-EDTA was used as a starting point, and was combined

with additional proteases to improve disaggregation. The majority of the combinations did not separate cell clumps efficiently. More than 90% of the cells were disaggregated with the combination of collagenase, DNase I and trypsin-EDTA, followed by mechanical pipetting.

**Table 4.1 Enzyme combinations and proportion of singular viable cells.**

| Enzymes | Mechanical pipetting | % (singular viable cells) |
|---|---|---|
| Trypsin-EDTA | Yes | 20%-50% |
| Trypsin-EDTA + DNase I | Yes | 50%-70% |
| DNase I | Yes | 20%-30% |
| Collagenase | Yes | 50%-70% |
| Collagenase + Trypsin-EDTA | Yes | 50%-70% |
| Collagenase + DNase I | Yes | 70%-80% |
| Collagenase +DNase I + trypsin-EDTA | Yes | >90% |

After 24 h culture in plates coated with poly-lysine, approximately 80% of the disaggregated OECs adhered to the bottom of each well. Cells remained singular with the original morphology and polarity (Figure 4.3a). With H&E staining, the shape of cell was spherical and cilia were still observed on the cell surfaces (Figure 4.4a).

For the *in vitro* assays, the initial OEC cell suspensions contained ciliated and nonciliated cells but by 24 h after seeding, free floating epithelial cells had formed vesicles with cilia on their external surface. After 48 h (for sheep) or 72 h (for cattle), some cells were adhered to the bottom of the flasks and began to form a small monolayer. Others were reassociated into aggregates with numerous ciliated cells at the periphery. These reassociated cell clusters exhibited rapid motility in the culture medium. When the culture medium was first changed on the $3^{th}$-$4^{th}$ day, the monolayer had extended across approximately 60% of the bottom of the wells. The ciliated activity was still observed on clumps of cells or on the top of some monolayers. By the $7^{th}$ day of culture, the cells had reached complete confluency (Figure 4.3b). With H&E staining, two cell populations were observed: a population of large flat and polygonal cells containing some vacuoles surrounded by a second population of smaller cells. Most of the cellular cytoplasm was occupied by a large ovoid nucleus (Figure 4.4b). Percentage of ovine cell viability tested by the exclusion of trypan blue was 87 ± 4.2%

(sheep) and 93 ± 2.9% (cattle) for the 24 h culture and 90 ± 2.3% (sheep) and 89 ± 2.7% (cattle) for the *in vitro* assay (Figure 4.2b).

### 4.1.2 Purity of the OEC cultures

The epithelial nature of these cells was confirmed by immunohistochemical analysis using monoclonal antibodies specific to the cytokeratins 8 and 18. In the order of 50% of the 24 h-old (Figure 4.5) and more than 95 % of the 7 day-old cells stained positive with these anti-cytokeratin antibodies (Figure 4.6) - typical of non-stratified epithelia. After 7 days of culture, the OOECs appeared to be considerably smaller than the BOECs. All nuclei of the 7 day-old cells were surrounded by a meshwork of stained filaments (Figure 4.6), while for 24 h-old cells; this meshwork could not be clearly observed (Figure 4.5). The control assay using only secondary antibody conjugated with fluorescence but with no primary antibody showed no signal.

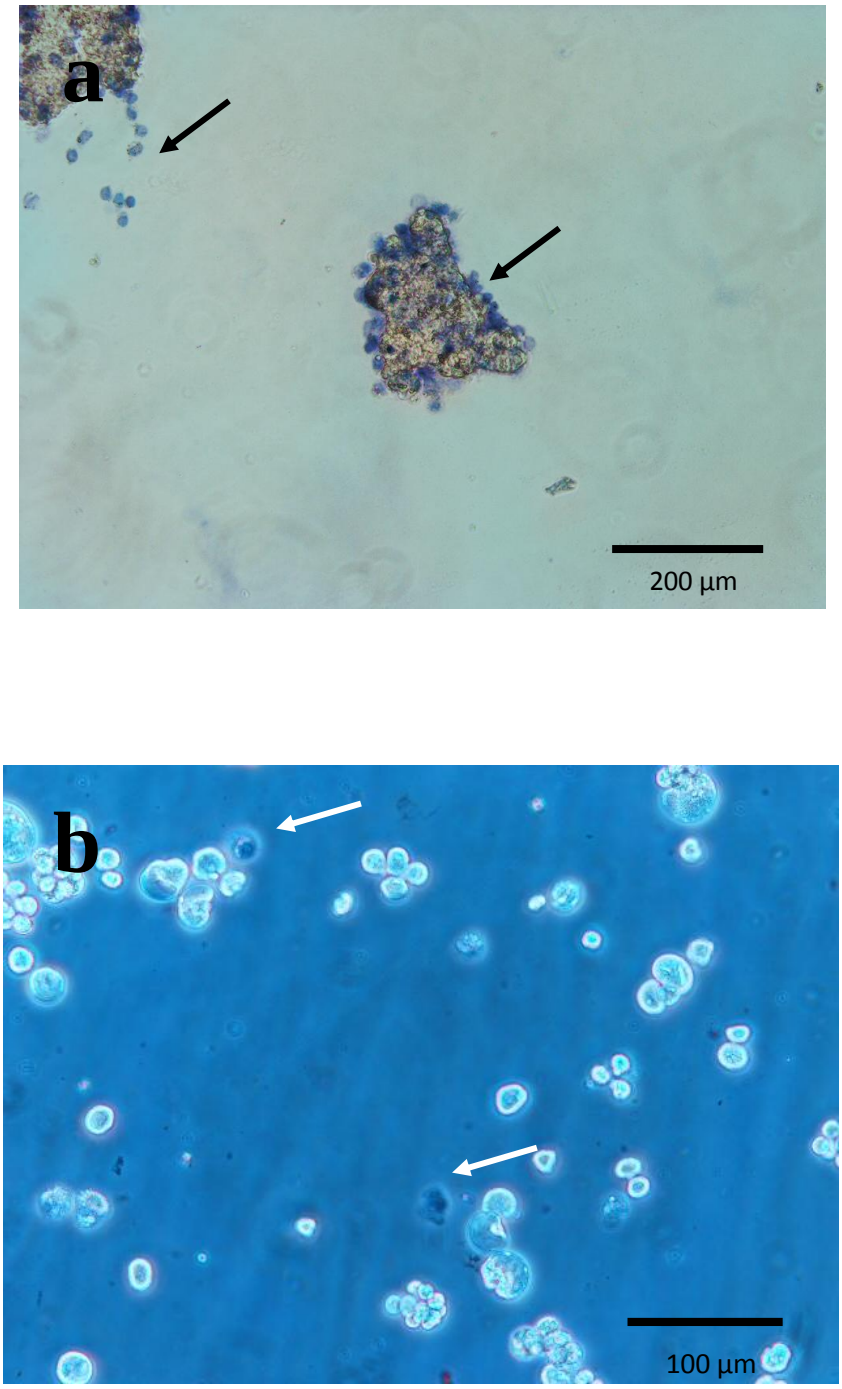
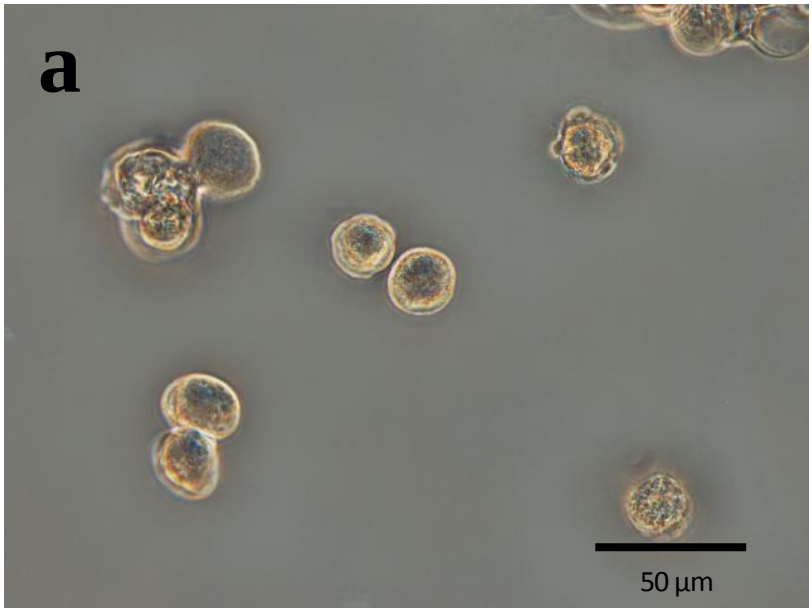



**Figure 4.2** **Evaluation of OOECs viability at 1 h after isolation (a) or on day 7 (b). Non-viable cells (arrows) are stained blue.**



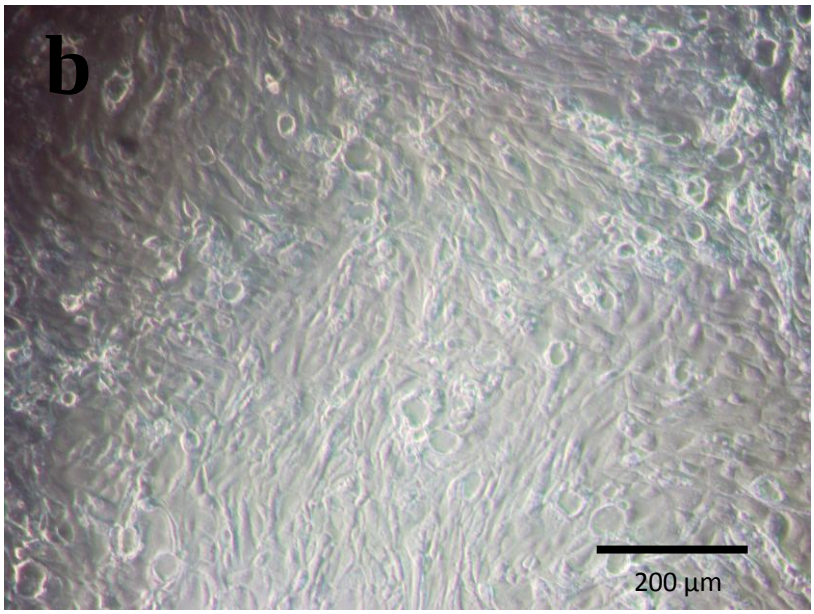




**Figure 4.3** **Phase-contrast micrograph of enzymatically dissociated OOECs in D-MEM (a) and confluent layer of cultured OOECs, after 7 days of culture (b) . where cilia on the surface are absent.**

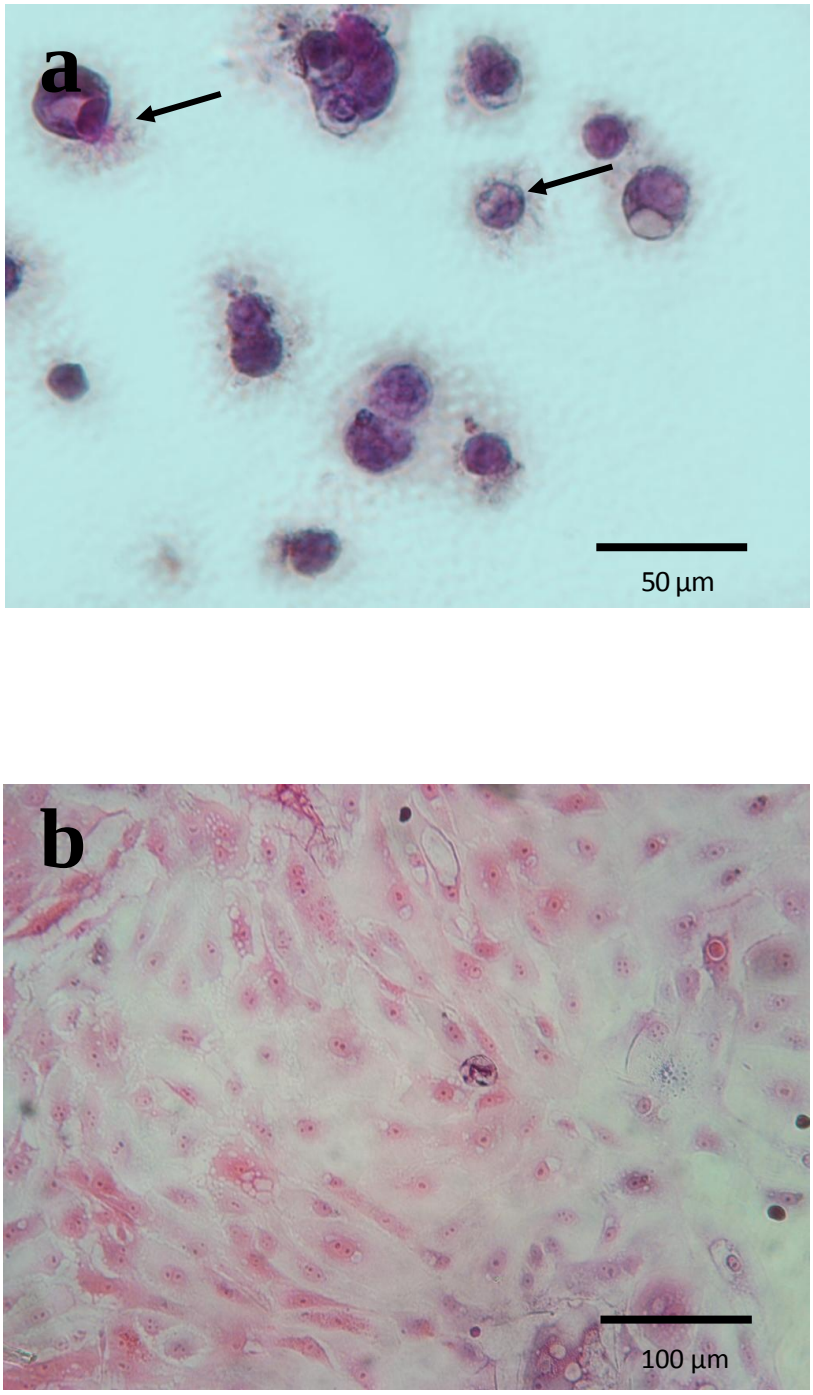



**Figure 4.4** **Micrograph of enzymatically dissociated OOECs (a) - cilia (arrows) can be observed on the apical membrane. Confluent monolayer of 7 day-old OOECs (b). H&E staining of both preparations.**

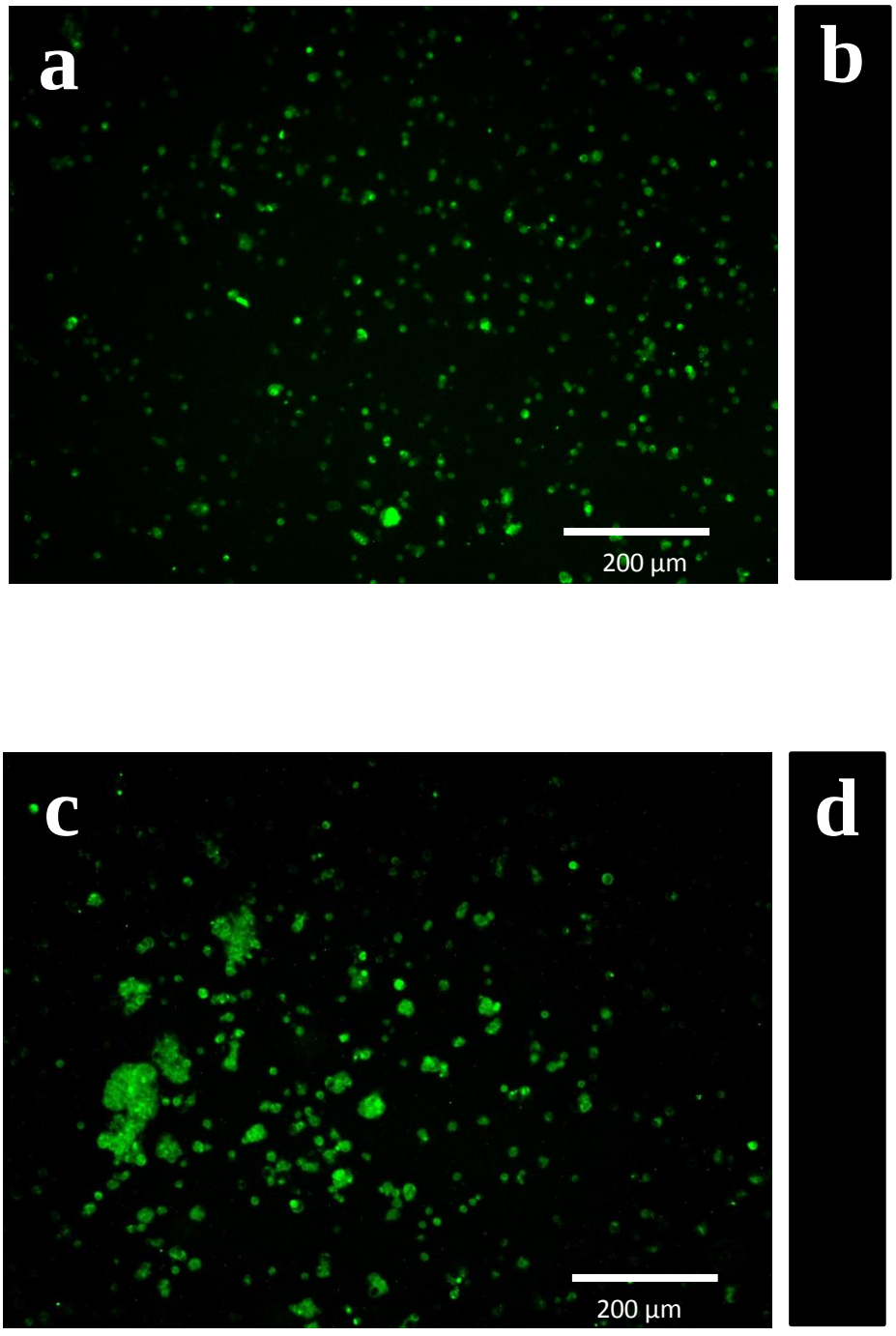



**Figure 4.5** **Micrograph of 24 h-old OECs following immunostaining for cytokeratin 8, 18 from (a) sheep and (c) cattle. Figure (b) and (d) are primary antibody blank controls.**

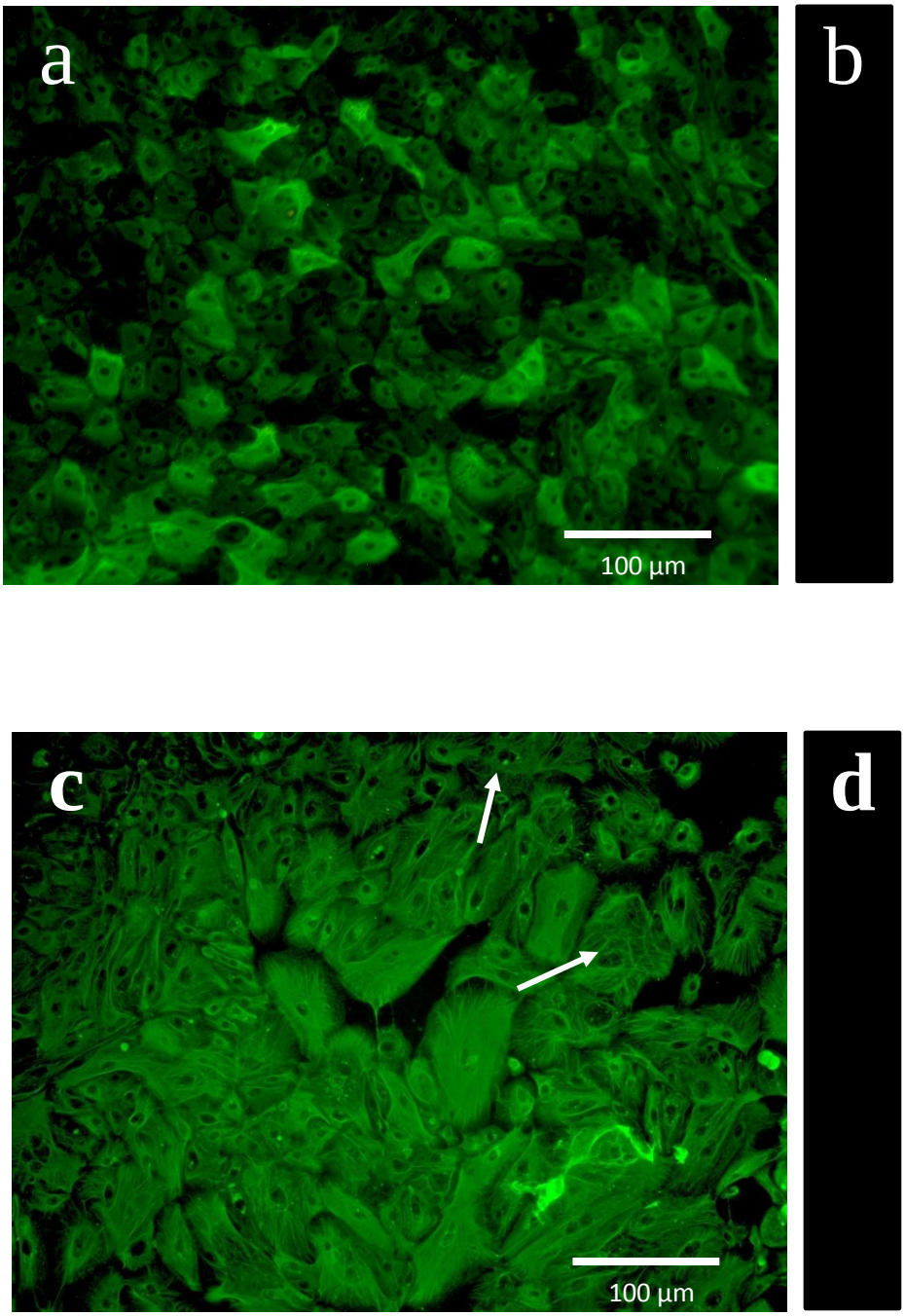



**Figure 4.6** **Micrograph of 7 day-old OECs following immunostaining for cytokeratin 8, 18 in (a) ovine and (c) bovine cells. Note the filaments (white arrows) in the cytoplasma. Figures (b) and (d) are primary antibody blank controls.**

## 4.2 Intestinal epithelial cell (IEC) cultures

### 4.2.1 Cell morphology, yield and viability

Following isolation, observations using light microscopy showed most epithelial cells were singular and suspended in the culture medium. The yield of harvested IECs from sheep ranged from 8.0-8.4 $\log_{10}$ cells in each 2 cm × 2 cm ileum section. Trypan blue showed cell viability was more than 90% (Figure 4.7).

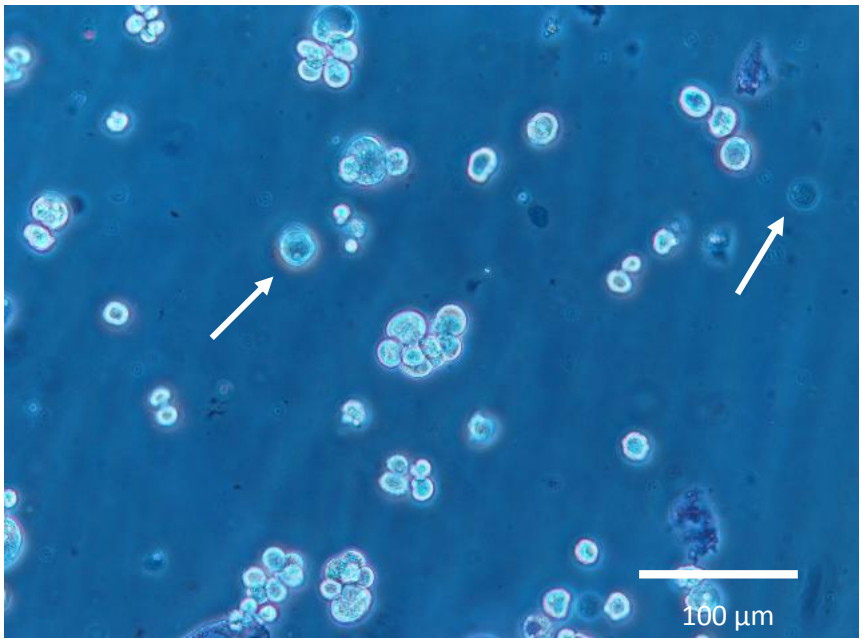
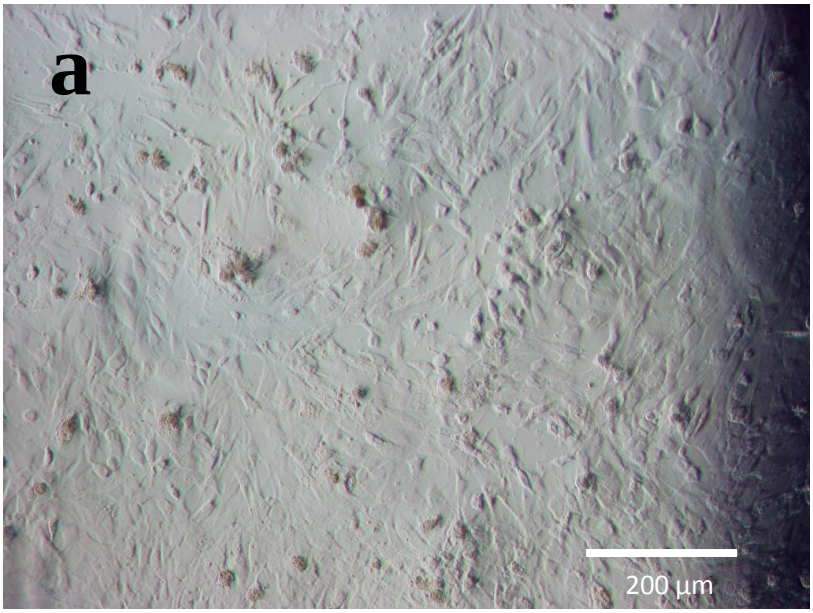


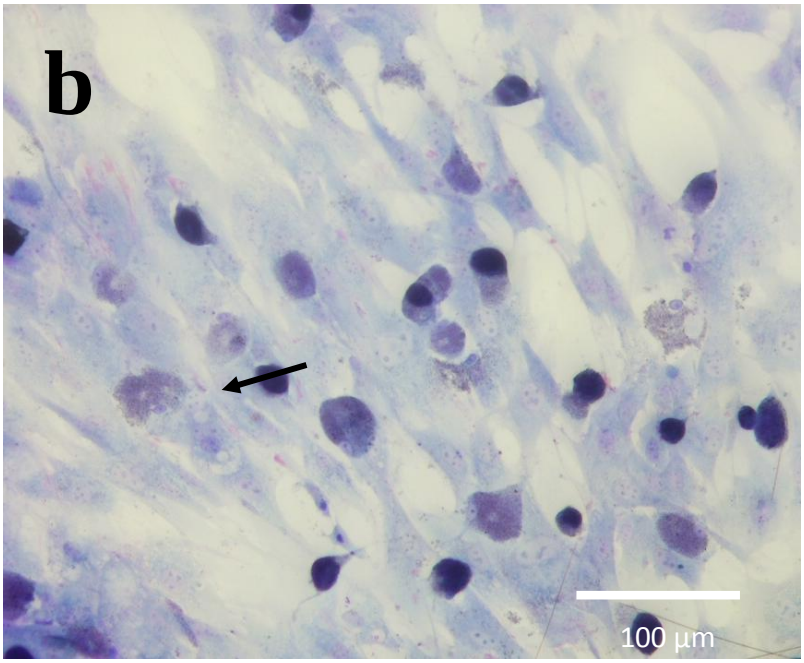

**Figure 4.7 OIEC viability after 1 h isolation where non-viable cells (arrows) are stained blue.**

For the *in vitro* assay, the isolated OIECs were cultured in D-MEM supplemented with 10% (v/v) FBS, 50 ng of EGF/ml, 8 µg of insulin/ml, 10 µg of gentamycin/ml, 50 µg of hydrocortisone/ ml, 100 µg of streptomycin/ml, 100 U of penicillin/ml, and 2.5 µg of amphotericin B/ml at 37$^{o}$C, with 5% $CO_2$. The cultures generally grew in 24-well plates coated with collagen; cells began to attach and spread over the surface after 72 h. When the culture media was first changed on the 3$^{th}$-4$^{th}$ day, approximately 40% of the bottom of the wells was covered by cells. Small cobblestone colonies extended and ultimately coalesced, forming a confluent monolayer by the 7$^{th}$ day (Figure 4.8a). With Giemsa staining two cell populations were observed. One population was spherical cells, while the other population was made up of large flat and polygonal cells (Figure 4.8b).

### 4.2.2 Purity of the IEC cultures

As with the OECs, cell purity was characterized by immunohistochemical analysis using antibodies against cytokeratins 8 and 18. Approximately 50%-60% of cells stained positive. The cells that stained positive were the spherical population, while the flat and polygonal cells were negative (Figure 4.8c). The control assay using only the secondary antibody conjugated with fluorescence showed no signal.



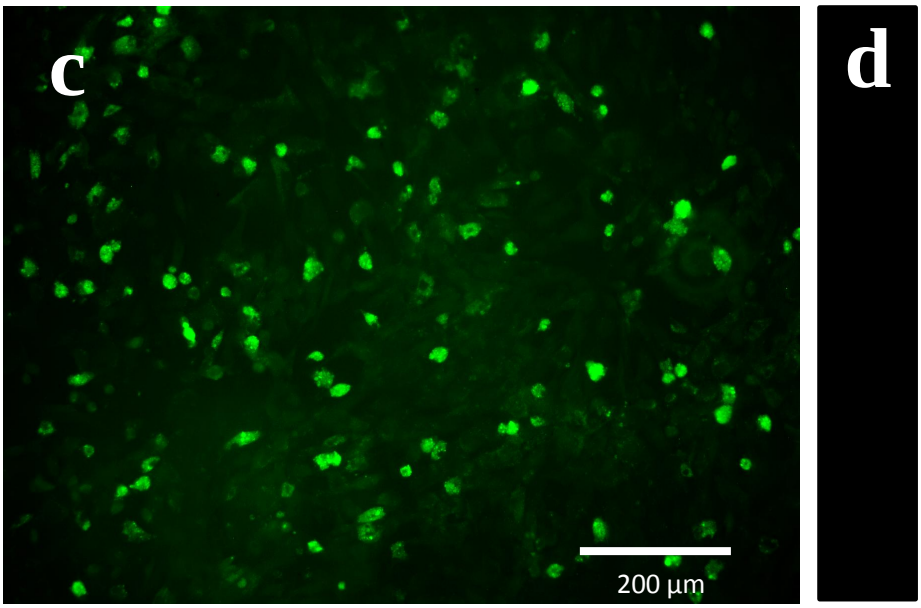



**Figure 4.8** **Phase-contrast micrograph of confluent layer of cultured OIECs after 7 days of culture (a). Confluent monolayer of 7 day-old OIECs with Giemsa staining (b). Immunostaining for cytokeratin 8, 18 (c). Figure (d) is primary antibody blank control.**

## 4.3 Optimisation of infection assays

### 4.3.1 Growth characteristics of *Salmonella* cultures

Following overnight (12-15 h) incubation at 25$^{o}$C, the *S.* Brandenburg strains were further grown in LB broth for 2, 4, 6, 8, 10, or 12 h, at 37$^{o}$C, in order to determine their growth curves. In general, the growth of *Salmonella* isolates was most rapid in the first 2 h with a growth plateau occurring after 4 h for 3684, 3062, 4468 and 4527 and 6 h for 1979 (Figure 4.9). The concentration of all these *Salmonella* isolates after 2 h were higher than 6.0 $\log_{10}$ CFUs per ml (OD=0.300), sufficient for the infectivity assays.

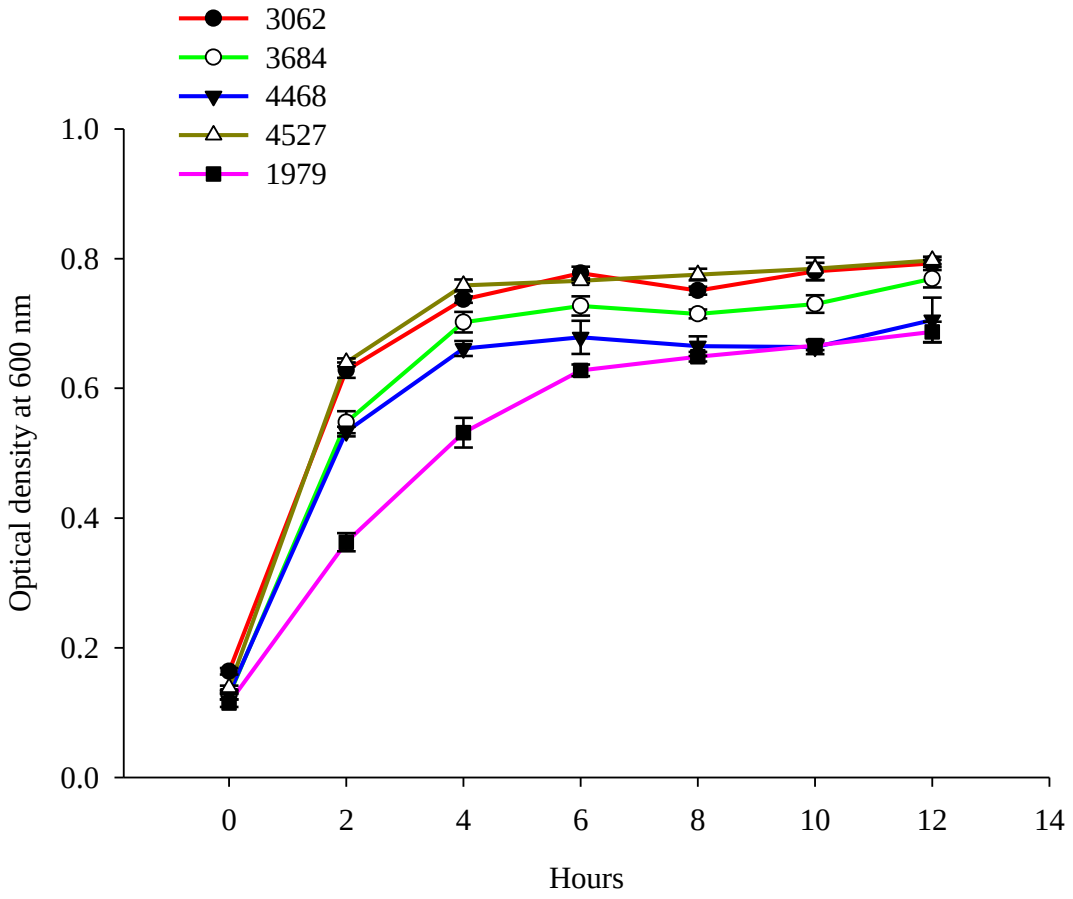



**Figure 4.9 Growth curves of *S.* Brandenburg isolates (3602, 3684, 4468, and 4527) and *S.* Typhimurium isolate (1979) with SD.**

### 4.3.2 Efficacy of gentamycin

Gentamycin (GIBCO) was tested for its ability to inhibit the growth of *S.* Brandenburg and *S.* Typhimurium isolates in cell culture media. All of the *S.* Brandenburg and *S.* Typhimurium strains were immediately inactivated after exposure to gentamycin *in vitro* and thereafter throughout the gentamycin protection assay.

### 4.3.3 Lytic buffer viability assay

The ability of sodium deoxycholate to lyse the OECs and its potential toxicity to *Salmonella* was tested. Treatment with 0.1% Sodium deoxycholate (Sigma) resulted in complete lysis of the OECs within 5 min as assessed by light microscopy. To test the potential toxicity of the lytic buffers, various *Salmonella* isolates were suspended in plain D-MEM medium or sodium deoxycholate for 30 min. Following lytic treatment to the *S.* Brandenburg and Typhimurium

isolates with 0.1% sodium deoxycholate there were similar recoveries to the D-MEM control with sodium deoxycholate (Table 4.2). Therefore, 0.1% sodium deoxycholate was chosen as a cell lytic buffer for the gentamycin protection assay.

**Table 4.2 Quantification of *Salmonella* viability after incubation in lytic buffer ($log_{10}$ [CFUs/ml]).**

| Isolates | Lytic Buffer | Bacterial Recovery (mean ± SD) | |
|---|---|---|---|
| | | 10 min | 30 min |
| 3684 | Media control | 12.7 (± 0.03) | 12.8 (± 0.01) |
| | 0.1% Sodium deoxycholate | 12.8 (± 0.04) | 12.8 (± 0.03) |
| 3062 | Media control | 12.9 (± 0.02) | 12.9 (± 0.02) |
| | 0.1% Sodium deoxycholate | 12.9 (± 0.01) | 12.9 (± 0.04) |
| 4468 | Media control | 12.9 (± 0.02) | 12.9 (± 0.01) |
| | 0.1% Sodium deoxycholate | 12.9 (± 0.04) | 12.9 (± 0.03) |
| 4527 | Media control | 12.9 ( ± 0.02) | 12.9 (± 0.01) |
| | 0.1% Sodium deoxycholate | 12.9 ( ± 0.02) | 12.9 (± 0.02) |
| 1979 | Media control | 12.7 (± 0.01) | 12.7 (± 0.02) |
| | 0.1% Sodium deoxycholate | 12.8 (± 0.01) | 12.8 (± 0.01) |

## 4.4 *Salmonella* infection assays

### 4.4.1 *Salmonella* infections in 24 h-old OOEC cultures

The infection characteristics of *S.* Brandenburg and *S.* Typhimurium were compared in OOECs cultured in a 24-well plate at approximately 5.0 log $_{10}$ cells per ml, in D-MEM containing 10% (v/v) FBS. To the cell cultures were added *S.* Brandenburg isolates (3062, 3684, 4468 and 4527) or *S.* Typhimurium isolate (1979) at a multiplicity of infection (MOI) of 1:20-40 (Appendix B.1, Figure 4.10). The infection process was divided into three parts; adhesion, invasion and replication, according to the stage of the bacterial infection.

#### 4.4.1.1 Media control

An estimate of the proliferative ability of bacteria in growth media without the presence of cells was made following the addition of *S.* Brandenburg (3684, 3062, 4468, and 4527) and *S.* Typhimurium (1979). Generally there was a little variation among the *Salmonella* isolates in the media control after 1 h incubation. The total number of *Salmonella* organisms varied from 6.88 (isolate 3062) $\log_{10}$ CFUs per ml to 6.78 (isolate 4527) $\log_{10}$ CFUs per ml. For each isolate, the variance was between 0.001 and 0.008 $\log_{10}$ CFUs (Table 4.3).

#### 4.4.1.2 Adhesion assay

After 1 h of incubation, the percentage of *Salmonella* that adhered to 24 h-old OOECs ranged from 0.2% (isolate 1979) to 1% (isolate 3684). That is, the number of adhered bacteria following washing of the cells divided by the numerical value of the same isolate after culture in media for 1 h. Isolate 3684 adhered at approximately 4.80 $\log_{10}$ CFUs per ml, followed in descending order by isolates 4468, 3062, 4527 and 1979. The variance of each isolate ranged from 0.007 (isolate 3062) to 0.100 (isolate 1979) $\log_{10}$CFUs per ml (Table 4.3).

#### 4.4.1.3 Invasion assay

As a separate assay, following 1 h incubation, 100 µg of gentamycin was added to the cell cultures and incubation proceeded for another 1 h to kill the extracellular organisms. As described in Figure 4.10, the number of bacteria invading the OOECs varied from 3.67 $\log_{10}$ CFUs per ml (isolate 3684) to 3.18 $\log_{10}$ CFUs per ml (isolate 4527). The percentage of bacteria that were intracellular divided by those originally adhered uptake ranged from 27% (isolate 1979) to 7% (isolate 3684). The variance of the triplicates of each isolate ranged from 0.002 (isolate 3684) to 0.172 (isolate 1979) $\log_{10}$ CFUs per ml (Table 4.3).

#### 4.4.1.4 Replication assay

The bacteria that had penetrated into cells with the addition of 100 µg of gentamycin were further incubated for 23 h to allow them to replicate. After 24 h incubation, the replication level of *Salmonella* isolates fluctuated between 5.02 $log_{10}$ CFUs per ml (isolate 3684) and 4.0 $log_{10}$ CFUs per ml (isolate 4527). The multiplication rate collected from replication number divided by invasion number varied from 20 to 22 fold (isolates 3062, 3684 and 4468) to 6 or 7 fold (isolates 1979, 4527). The variance of triplicates of each isolate ranged from 0.198 (isolate 3684) to 0.394 (4468) $log_{10}$ CFUs per ml (Table 4.3).

**Table 4.3 Means and variances of media control, adhesion, invasion, and replication of *Salmonella* in 24 h-old OOEC cultures ($log_{10}$ [CFUs/ml]).**

| OOECs, 24 h, 10% FBS | | | | |
|---|---|---|---|---|
| **Isolates** | **Media control** | **Adhesion** | **Invasion** | **Replication** |
| 3062 | 6.88 (0.001) | 4.56 (0.007) | 3.60 (0.016) | 4.91 (0.271) |
| 3684 | 6.80 (0.005) | 4.80 (0.076) | 3.67 (0.002) | 5.02 (0.198) |
| 4468 | 6.80 (0.005) | 4.62 (0.008) | 3.64 (0.020) | 4.98 (0.394) |
| 4527 | 6.78 (0.005) | 4.30 (0.035) | 3.18 (0.029) | 4.00 (0.284) |
| 1979 | 6.82 (0.008) | 4.21 (0.100) | 3.64 (0.172) | 4.42 (0.386) |

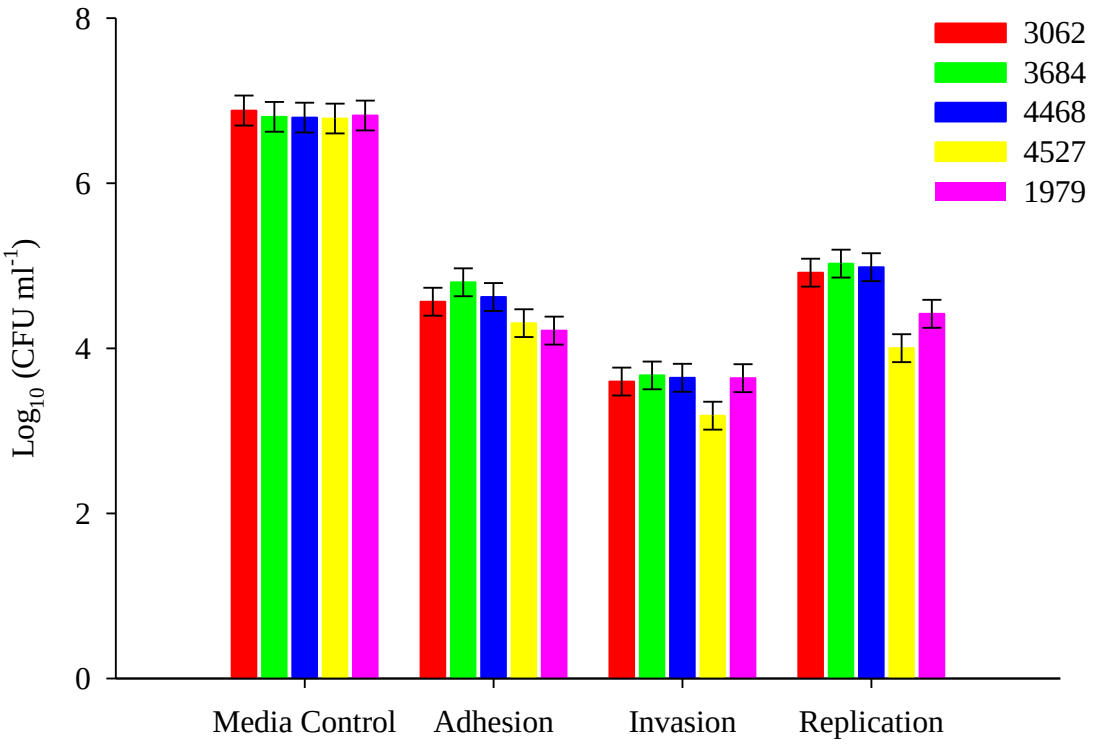



**Figure 4.10 Media control, adhesion, invasion and replication of *S.* Brandenburg and *S.* Typhimurium isolates in 24 h-old OOEC cultures, with SEM bars (in triplicate).**

#### 4.4.1.5 Student *t*-test comparisons of adhesion, invasion and replication

Adhesion, invasion and replication abilities of the *Salmonella* isolates over 24 h in the OOECs were based on the means of the triple measurements of 3 samples for 3 separate experiments, and were compared among each other using the Student *t*-test (Table 4.4, Appendix C.1 and D.1).

There were no significant differences between the *S.* Brandenburg isolates 3062, 3684, and 4468. The adhesion of *S.* Brandenburg isolates (3062, 3684 and 4468) was higher than *S.* Typhimurium isolate 1979 ($P<0.01$), and isolates 3684, and 4468 adhered more than isolate 4527 ($P<0.01$).

The results of the invasion assay indicated that *S.* Brandenburg isolate 4527 invaded significantly less than *S.* Brandenburg and *S.* Typhimurium isolates 3062, 3684, 4468, 1979 ($P<0.01$), but there was no difference between the *S.* Brandenburg isolates 3062, 3684, and 4468 and *S.* Typhimurium isolate 1979.

The replication of *S.* Brandenburg isolate 4527 was significantly lower than isolates 3062, 3684, 4468 and 1979 ($P<0.01$). The replication of *S.* Brandenburg isolates 3062, 3684, 4468 was statistically higher than *S.* Typhimurium isolate 1979 ($P<0.01$). There were no significant differences between *S.* Brandenburg isolates 3062, 3684, 4468.

**Table 4.4 Student *t*-test values for adhesion, invasion and replication of *Salmonella* in 24 h-old OOEC cultures.**

| Isolates | Adhesion | Invasion | Replication |
|---|---|---|---|
| 3062 *vs* 3684 | -1.77 | -0.56 | -0.83 |
| 3062 *vs* 4468 | -0.42 | -0.34 | -0.50 |
| 3062 *vs* 4527 | 1.97 | 3.14* | 6.92* |
| 3062 *vs* 1979 | 2.65* | -0.31 | 3.77* |
| 3684 *vs* 4468 | 1.35 | 0.22 | 0.33 |
| 3684 *vs* 4527 | 3.74* | 3.70* | 7.75* |
| 3684 *vs* 1979 | 4.42* | 0.25 | 4.60* |
| 4468 *vs* 4527 | 2.39* | 3.48* | 7.42* |
| 4468 *vs* 1979 | 3.08* | 0.03 | 4.27* |
| 4527 *vs* 1979 | 0.68 | -3.45* | -3.15* |

***** represents significant differences between isolates at 99% confidence interval

### 4.4.2 *S.* Brandenburg infection in 24 h-old OOEC and BOEC cultures

This assay was focused on the host specificity of *S.* Brandenburg; OOECs and BOECs were cultured for 24 h in 24-well plates at approximately 5.0 log $_{10}$ cells per ml in complete D-MEM, containing 20% FBS. To the cell cultures was added the epidemic *S.* Brandenburg isolate 3684 at 1:20-40 MOI (Appendix B.2, Figure 4.11).

#### 4.4.2.1 Media control

When *Salmonella* isolate (3684) was cultured in D-MEM without cells, a two to four fold multiplication of bacteria usually occurred (Appendix B2), which gave a final concentration of 6.66 $\log_{10}$ CFUs per ml for the ovine cell assay and 6.80 $\log_{10}$ CFUs per ml for the bovine cell assay. The variance in ovine cells was 0.002 $\log_{10}$ CFUs per ml as compared to 0.009 $\log_{10}$ CFUs per ml in bovine cells (Table 4.5).

#### 4.4.2.2 Adhesion assay

After 1 h of incubation, 4.76 $\log_{10}$ CFUs per ml of bacteria adhered to the OOECs as compared to 4.51 $\log_{10}$ CFUs per ml in the BOECs cultures. The proportion of bacterial adhesion, that is, the number of adhered bacteria following washing of cells divided by the numerical value after culture in media for 1 h, was 1.3% (ovine cells) compared to 0.5% (bovine cells). The variance in ovine cells was 0.005 $\log_{10}$ CFUs per ml as compared to 0.035 $\log_{10}$ CFUs per ml in bovine cells (Table 4.5).

#### 4.4.2.3 Invasion assay

With the addition of gentamycin, 3.39 $\log_{10}$ CFU per ml of bacteria invaded OOECs as compared to 3.60 $\log_{10}$ CFUs per ml of bacteria that were internalized in the BOECs. That is, 4% of bacteria invaded OOECs, as compared to 12% in the BOEC cultures. The variance in ovine cells was 0.01 $\log_{10}$ CFUs per ml as compared to 0.081 $\log_{10}$ CFUs per ml in bovine cells (Table 4.5).

#### 4.4.2.4 Replication assay

After 24 h incubation, 5.14 $\log_{10}$ CFUs per ml of bacteria had replicated in OOECs as compared to 4.27 $\log_{10}$ CFUs per ml in BOEC. That is, replication frequencies of 56 fold in OOECs versus 5 fold in BOECs. The variance in ovine cells was 0.012 $\log_{10}$ CFUs per ml as compared to 0.182 $\log_{10}$ CFUs per ml in bovine cells (Table 4.5).

**Table 4.5** **Means and variances of media control, adhesion, invasion, and replication of *S.* Brandenburg in 24 h-old OOEC and BOEC cultures ($log_{10}$ [CFUs/ml]).**

| ***S.* Brandenburg isolate 3684, 20% FBS** | | |
|---|---|---|
| **Stage** | **Sheep** | **Cattle** |
| Media control | 6.66 (0.002) | 6.8 (0.009) |
| Adhesion | 4.76 (0.005) | 4.51 (0.035) |
| Invasion | 3.39 (0.01 ) | 3.60 (0.081) |
| Replication | 5.14 (0.012) | 4.27 (0.182) |

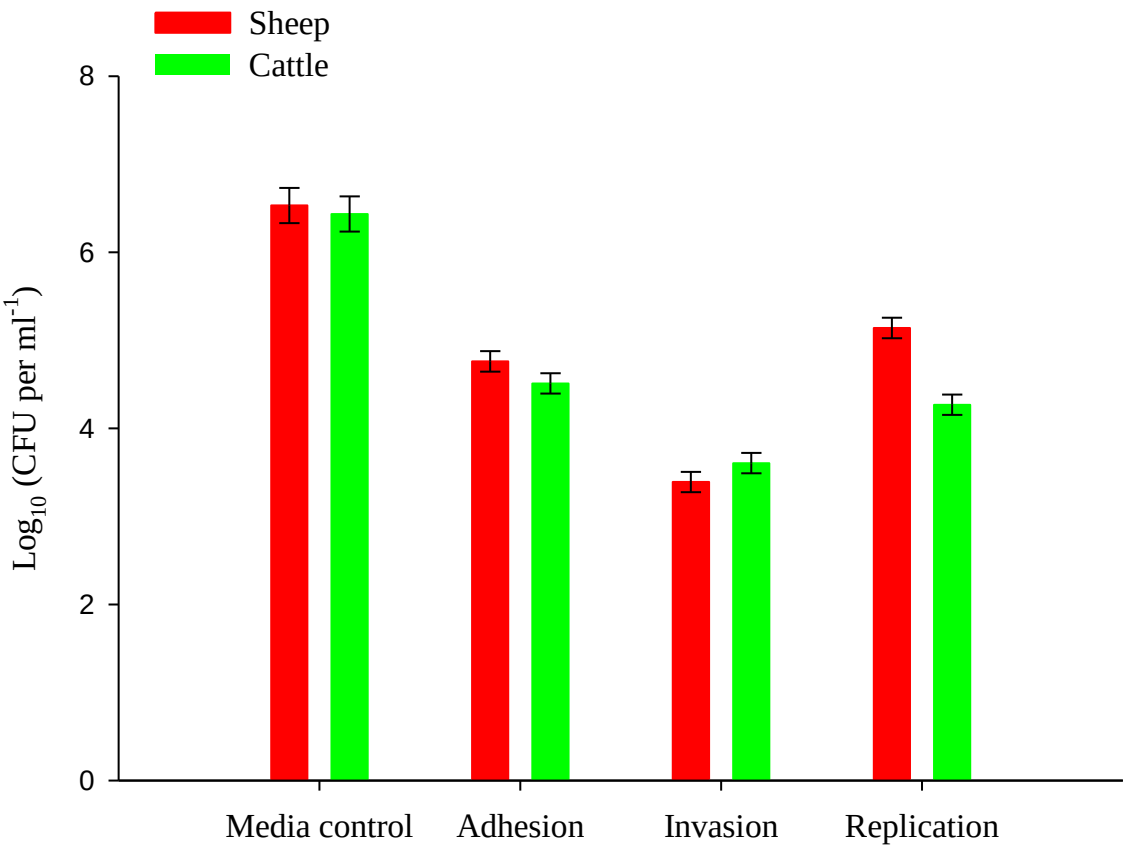



**Figure 4.11** **Media control, adhesion, invasion and replication of *S.* Brandenburg in 24 h-old OOEC and BOEC cultures, with SEM bars (in triplicate).**

#### 4.4.2.5 Student *t*-test comparison of adhesion, invasion and replication

Adhesion, invasion and replication abilities of the *Salmonella* isolate 3684 over 24 h in the OOEC and BOECs were based on the means of the triple measurements of 3 samples for 3 separate experiments, and were compared among each other using the Student *t*-test (Table 4.6, Appendix C.2 and D.2).

**Table 4.6 Student *t*-test values for adhesion, invasion and replication of *S.* Brandenburg in 24 h-old OOEC and BOEC cultures.**

| Species | Adhesion | Invasion | Replication |
|---|---|---|---|
| Sheep *vs* Cattle | 4.56* | -3.96* | 15.91* |

* represents significant differences between species at 99% confidence interval

The results indicate that *S.* Brandenburg isolate 3684 adhered to and replicated within OOECs more than BOECs ($P<0.01$). However, invasion of the bacteria in ovine cells was less than in bovine cells ($P<0.01$).

### 4.4.3 *Salmonella* infection in 7 day-old OOEC cultures

As with *ex vivo* assays, cells were cultured in a 24-well plate at approximately 5.0 log $_{10}$ cells per ml in D-MEM containing 10% (v/v) FBS. To the cell cultures were added *S.* Brandenburg isolates (3062, 3684, 4468 and 4527) or *S.* Typhimurium isolate (1979) at a MOI of 1:20-40 (Appendix B.3, Figure 4.12).

#### 4.4.3.1 Media control

An estimate of the proliferative ability of bacteria in growth media without the presence of cells was made following the addition of *S.* Brandenburg (3684, 3062, 4468, and 4527) and *S.* Typhimurium (1979). The number of *Salmonella* isolates organisms 1 h incubation in media varied from 6.56 (isolate 4468) $\log_{10}$ CFUs per ml to 7.18 (isolate 4527) $\log_{10}$ CFUs per ml. For each isolate, the variance was between 0.029 and 0.115 $\log_{10}$ CFUs per ml (Table 4.7).

#### 4.4.3.2 Adhesion assay

After 1 h of incubation, the percentage of *Salmonella* that adhered to 7 day-old OOECs ranged from 2% (isolate 4527) to 13% (isolate 1979). That is, the number of adhered bacteria following the washing of cells divided by the numerical value of the same isolate after culture in media for 1h. Isolate 1979 adhered at approximately 6.04 $\log_{10}$ CFUs per ml, which was the highest amount followed in descending order by isolates 3684, 3062, 4468 and 4527. The variance of each isolate ranged from 0.014 (isolate 4527) to 0.259 (isolate 1979) $\log_{10}$ CFUs per ml (Table 4.7).

#### 4.4.3.3 Invasion assay

As a separate assay, following 1 h incubation, 100 µg of gentamycin was added to the cell cultures and incubation proceeded for another 1 h to kill the extracellular organisms. As described in Figure 4.12, the number of bacteria invading the OOECs varied from 5.21 $\log_{10}$ CFUs per ml (isolate 1979) to 5.13 $\log_{10}$ CFUs per ml (isolate 3062 and 3684). The percentage of bacteria that were intracellular divided by those originally adhered ranged from 30% (isolate 3062) to 13% (isolate 4527). The variance of the triplicates of each isolate ranged from 0.005 (isolate 4527) to 0.12 (isolate 4468) $\log_{10}$ CFUs per ml (Table 4.7).

#### 4.4.3.4 Replication assay

The bacteria that had penetrated into cells with the addition of 100 µg of gentamycin were further incubated for 23 h to allow them to replicate. After 24 h incubation, the replication level of *Salmonella* isolates fluctuated between 6.13 $\log_{10}$ CFUs per ml (isolate 1979) and 5.73 $\log_{10}$ CFUs per ml (isolate 3062). The number of replicated bacteria divided by the invasion number varied from 4 fold (isolates 3684, 3062) to 18 fold (isolate 4527). The variance of triplicates of each isolate ranged from 0.005 (isolate 4527) to 0.183 (3684) $\log_{10}$ CFUs per ml (Table 4.7).

**Table 4.7 Means and variances of media control, adhesion, invasion, and replication of *Salmonella* in 7 day-old OOEC cultures ($\log_{10}$ [CFUs/ml]).**

| OOECs, 7 day, 10% FBS | | | | |
|---|---|---|---|---|
| **Isolates** | **Media control** | **Adhesion** | **Invasion** | **Replication** |
| 3062 | 6.68 (0.029) | 5.66 (0.032) | 5.13 (0.078) | 5.73 (0.122) |
| 3684 | 6.70 (0.060) | 5.75 (0.050) | 5.13 (0.086) | 5.74 (0.183) |
| 4468 | 6.56 (0.069) | 5.49 (0.072) | 4.94 (0.120) | 6.16 (0.100) |
| 4527 | 7.18 (0.077) | 5.45 (0.014) | 4.56 (0.005) | 5.82 (0.005) |
| 1979 | 6.94 (0.115) | 6.04 (0.259) | 5.21 (0.025) | 6.13 (0.010) |

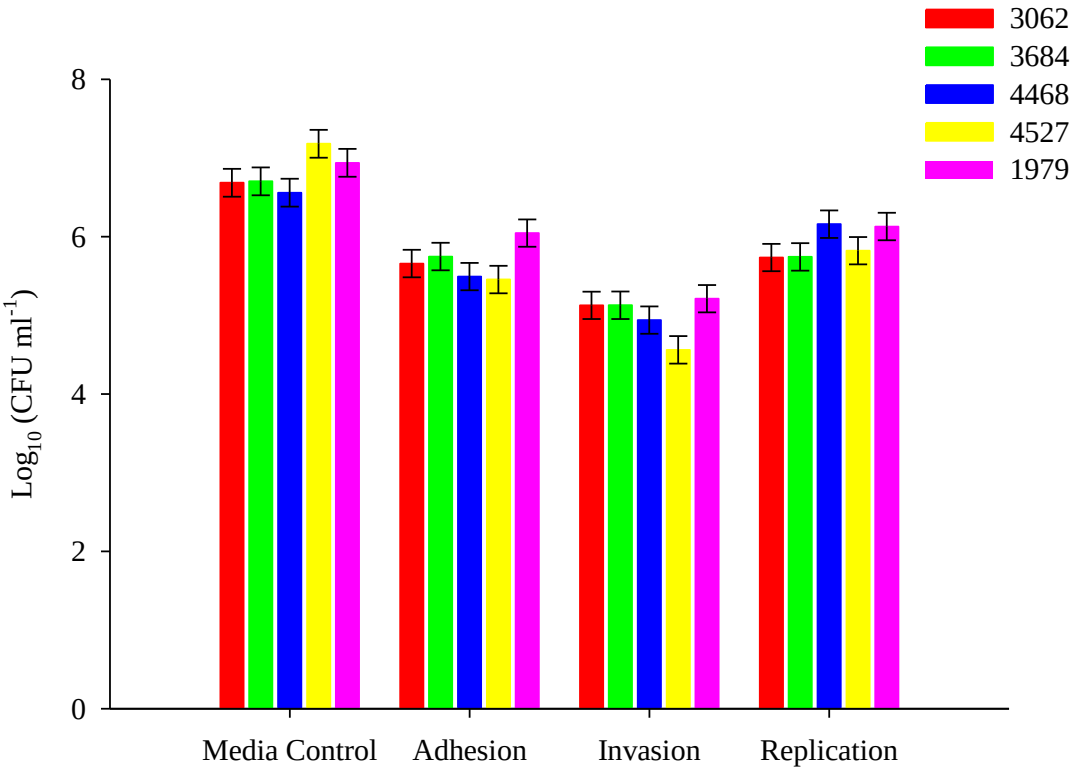



**Figure 4.12 Media control, adhesion, invasion and replication of *S.* Brandenburg and *S.* Typhimurium in 7 day-old OOEC culture, with SEM bars (in triplicate).**

#### 4.4.3.5 Student *t*-test comparisons of adhesion, invasion and replication

Adhesion, invasion and replication abilities of the *Salmonella* isolates in the 7 day-old OOECs were based on the means of the triple measurements of 3 samples for 3 separate experiments which were compared among each other using the Student *t*-test (Table 4.8, Appendix C.3 and D.3).

The adhesion of *S.* Typhimurium isolate 1979 was greater than *S.* Brandenburg isolates 3062, 3684, 4468, and 4527 ($P<0.01$).

The invasion of *S.* Brandenburg isolate 3062, 3684, 4468 and *S.* Typhimurium isolate 1979 was significantly higher than the *S.* Brandenburg isolate 4527 ($P<0.01$). *S.* Typhimurium isolate 1979 invaded more than *S.* Brandenburg isolate 4468 ($P<0.01$).

The replication of 4468 and 1979 were significantly more than 3062, 3684 and 1979 ($P<0.01$). However, there were no significant differences between isolate 3062, 3684, and

4527 and *S.* Brandenburg isolate 4468 replicated at a similar rate to *S.* Typhimurium isolate 1979.

**Table 4.8 Student *t*-test values for adhesion, invasion and replication of *Salmonella* in 7 day-old OOEC cultures.**

| Isolates | Adhesion | Invasion | Replication |
|---|---|---|---|
| 3062 *vs* 3684 | -0.81 | -0.01 | -0.08 |
| 3062 *vs* 4468 | 1.5 | 1.7 | -3.86* |
| 3062 *vs* 4527 | 1.85 | 5.15* | -0.8 |
| 3062 *vs* 1979 | -3.52* | -0.76 | -3.59* |
| 3684 *vs* 4468 | 2.31 | 1.71 | -3.78* |
| 3684 *vs* 4527 | 2.65* | 5.15* | -0.72 |
| 3684 *vs* 1979 | -2.71* | -0.75 | -3.51* |
| 4468 *vs* 4527 | 0.35 | 3.45* | 3.06* |
| 4468 *vs* 1979 | -5.02* | -2.46* | 0.27 |
| 4527 *vs* 1979 | -5.36* | -5.91* | -2.79* |

***** represents significant differences between isolates at 99% confidence interval

### 4.4.4 *S.* Brandenbur*g* infections in 7 day-old OOEC and BOEC cultures

For the *ex vivo* assays, OOEC and BOECs were cultured for 24 h in 24-well plates at approximately 5.0 log $_{10}$ cells per ml in complete D-MEM containing 20% FBS. To the cell cultures was added the epidemic *S.* Brandenburg isolate 3684 at 1:20-40 MOI (Appendix B.4, Figure 4.13).

#### 4.4.4.1 Media control

When *Salmonella* isolate (3684) was cultured in D-MEM without cells, a two to four fold multiplication of bacteria usually occurred (Appendix B4), which gave a final concentration of 6.67 $\log_{10}$ CFUs per ml for ovine cell assay and 7.02 $\log_{10}$ CFUs per ml for bovine cell assay. The variance in ovine cells was 0.126 $\log_{10}$ CFUs per ml as compared to 0.06 $\log_{10}$ CFUs per ml in bovine cells (Table 4.9).

#### 4.4.4.2 Adhesion assay

After 1 h of incubation, approximately 5.59 $\log_{10}$ CFUs per ml of bacteria adhered to the OOECs as compared to 5.64 $\log_{10}$ CFUs per ml in BOECs cultures. The proportion of bacterial adhesion, that is, the number of adhered bacteria following washing of cells divided by the numerical value after culture in media for 1 h, was 7% (ovine cells) compared to 4% (bovine cells). The variance in ovine cells was 0.007 $\log_{10}$ CFUs per ml as compared to 0.015 $\log_{10}$ CFUs per ml in bovine cells (Table 4.9).

#### 4.4.4.3 Invasion assay

With the addition of gentamycin, 5.44 $\log_{10}$ CFU per ml of bacteria invaded OOECs as compared to 5.47 $\log_{10}$ CFUs per ml of bacteria that were internalized in the BOECs. That is, 71% of bacteria invaded OOECs, as compared to 68% in the BOEC cultures. The variance in ovine cells was 0.005 $\log_{10}$ CFUs per ml as compared to 0.021 $\log_{10}$ CFUs per ml in bovine cells (Table 4.9).

#### 4.4.4.4 Replication assay

After 24 h incubation, 6.13 $\log_{10}$ CFUs per ml of bacteria were replicated in OOECs as compared to 5.48 $\log_{10}$ CFUs per ml in BOECs. That was a replication frequency of 5 fold in OOECs versus 1 fold in BOECs. The variance in ovine cells was 0.269 $\log_{10}$ CFUs per ml as compared to 0.028 $\log_{10}$ CFUs per ml in bovine cells (Table 4.9).

**Table 4.9** **Mean and variances of media control, adhesion, invasion and replication of *S.* Brandenburg in 7 day-old OOEC and BOEC cultures ($log_{10}$ [CFUs/ml]).**

| ***S.* Brandenburg isolate 3684, 20% FBS** | | |
|---|---|---|
| **Stage** | **Sheep** | **Cattle** |
| Media control | 6.77 (0.126) | 7.02 (0.060) |
| Adhesion | 5.59 (0.007) | 5.64 (0.015) |
| Invasion | 5.44 (0.005) | 5.47 (0.021) |
| Replication | 6.13 (0.269) | 5.48 (0.028) |

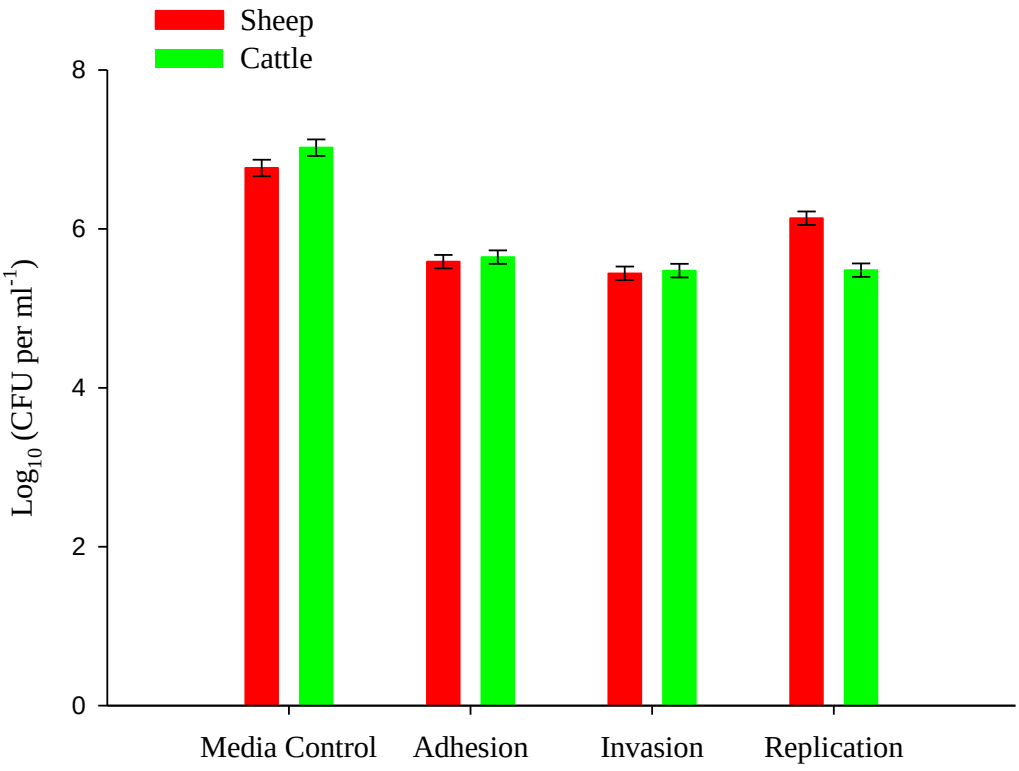



**Figure 4.13** **Media control, adhesion, invasion and replication of *S.* Brandenburg in 7 day-old OOEC and BOEC cultures, with SEM bars (in triplicates).**

#### 4.4.4.5 Student *t*-test comparison of adhesion, invasion and replication

Adhesion, invasion and replication abilities of the *Salmonella* isolate 3684 in the 7 day-old OOECs and BOECs were based on the means of the triple measurements of 3 samples for 3 separate experiments and were compared between each other using the Student *t*-test (Table 4.10, Appendix C.4 and D.4).

**Table 4.10 Student *t*-test values for adhesion, invasion and replication of *S.* Brandenburg in 7 day-old OOEC and BOEC cultures.**

| Species | Adhesion | Invasion | Replication |
|---|---|---|---|
| Sheep *vs* Cattle | -1.02 | -0.61 | 11.68* |

***** represents significant differences between species at 99% confidence interval

The result of *t*-test (Replication) showed that replication of *S.* Brandenburg was significantly more in OOEC, as compared to BOEC cultures ($P<0.01$). However, there were no significant differences between sheep and cattle for adhesion and invasion.

### 4.4.5 *Salmonella* infections in 7 day-old OOEC and OIEC cultures

This assay was to test the tissue tropism of *S.* Brandenburg. OIECs and OOECs were routinely cultured in a 24-well plate at approximately 5.0 log $_{10}$ cells per ml in D-MEM containing 10% FBS. To the cell cultures was added *S.* Brandenburg isolate 3684 at an approximate MOI of 1:20-40 (Appendix B.5, Figure.4.14). As with the previous assays, the process of infection was divided into adhesion, invasion and replication.

#### 4.4.5.1 Media control

When *Salmonella* isolate (3684) was cultured in D-MEM without cells, a two to four fold multiplication of bacteria usually occurred (Appendix B5), which gave a final concentration of 6.78 $\log_{10}$ CFUs per ml for OIECs assay and 6.70 $\log_{10}$ CFUs per ml for OOECs assay. The variance in OIECs was 0.005 $\log_{10}$ CFUs per ml as compared to 0.06 $\log_{10}$ CFUs per ml in OOECs (Table 4.11).

#### 4.4.5.2 Adhesion assay

After 1 h of incubation, approximately 5.63 $\log_{10}$ CFUs per ml of bacteria adhered to the OIECs as compared to 5.75 $\log_{10}$ CFUs per ml in OOECs cultures. The proportion of bacterial adhesion, that is the number of adhered bacteria following washing of cells divided by the numerical value after culture in media for 1 h, was 7% (OIEC cultures) compared to 11% (OOEC cultures). The variance in OIEC cultures was 0.002 $\log_{10}$ CFUs per ml as compared to 0.050 $\log_{10}$ CFUs per ml in OOECs (Table 4.11).

#### 4.4.5.3 Invasion assay

With the addition of gentamycin, 4.34 $\log_{10}$ CFU per ml of bacteria invaded OIECs as compared to 5.13 $\log_{10}$ CFUs per ml of bacteria that were internalized in the OOECs. That was 5% of bacteria invaded OIECs, as compared to 24% in the OOEC cultures. The variance in OIEC cultures was 0.013 $\log_{10}$ CFUs per ml as compared to 0.087 $\log_{10}$ CFUs per ml in OOECs cultures (Table 4.11).

#### 4.4.5.4 Replication assay

After 24 h incubation, 5.43 $\log_{10}$ CFUs per ml of bacteria were replicated in OIECs as compared to 5.74 $\log_{10}$ CFUs per ml in OOECs. That was a replication frequency of 12 fold

in OIECs versus 4 fold in OOECs. The variance in OIECs was 0.018 $log_{10}$ CFUs per ml as compared to 0.183 $log_{10}$ CFUs per ml in OOECs (Table 4.11).

**Table 4.11** **Means and variances of media control, adhesion, invasion and replication of *S.* Brandenburg in 7 day-old OIEC and OOEC cultures ($log_{10}$ [CFUs/ml]).**

| ***S.* Brandenburg isolate 3684, 10% FBS** | | |
|---|---|---|
| **Stage** | **OIEC** | **OOEC** |
| Media control | 6.78 (0.005) | 6.7 (0.060) |
| Adhesion | 5.63 (0.002) | 5.75 (0.050) |
| Invasion | 4.34 (0.013) | 5.13 (0.087) |
| Replication | 5.43 (0.018) | 5.74 (0.183) |

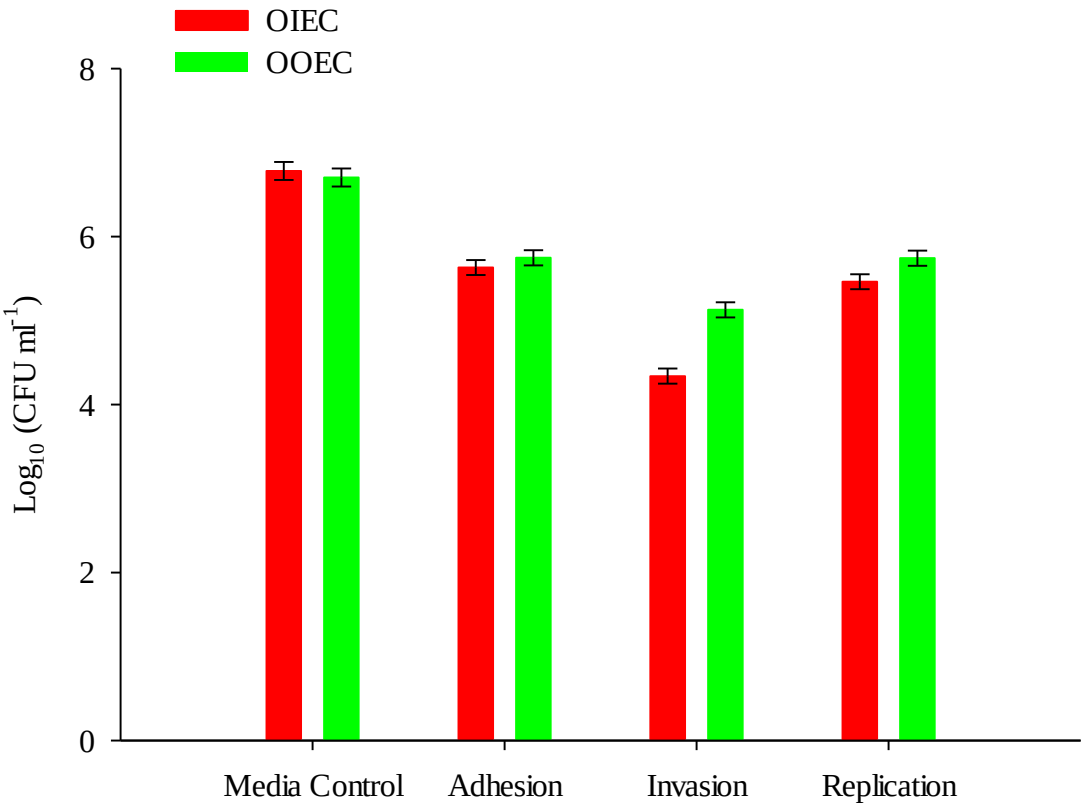



**Figure 4.14** **Adhesion, invasion and replication of *S.* Brandenburg in 7 day-old OIEC and OOEC cultures, with SEM bars (in triplicate).**

#### 4.4.5.5 Student *t*-test comparison of adhesion, invasion and replication

Adhesion, invasion and replication abilities of the *Salmonella* isolate 3684 in the 7 day-old OIECs and OOECs were based on the means of the triple measurements of 3 samples for 3 separate experiments, and were compared among each other using the Student *t*-test (Table 4.12, Appendix C.5 and D.5).

**Table 4.12 Student *t*-test values for adhesion, invasion and replication of *S.* Brandenbur*g* in 7 day-old OIEC and OOEC cultures.**

| Cell lines | Adhesion | Invasion | Replication |
|---|---|---|---|
| OIEC *vs* OOEC | -1.90- | -12.93* | -5.03* |

* represents significant differences between cell lines at 99% confidence interval

The results of *t*-test (Invasion and Replication) showed that invasion and replication of the isolate 3684 in OOECs were greater than in OIECs ($P<0.01$). However, there was no significant difference between OIECs and OOECs for adhesion.

# Chapter 5
# Discussion and conclusion

## 5.1 Infection assays

The virulence strategy common to *Salmonella* is to adhere, invade and multiply in the host cells. Establishment of these processes relies on virulence factors which are responsible for the pathogencity of *Salmonella* (Baumler, et al., 2000) and defence mechanisms exhibited by the host animal (Wray & Wray, 2000). Yet to date, little work has been done to investigate these differences in infective ability, host specificity and tissue tropism.

### 5.1.1 Infection differences between *S.* Brandenburg isolates in OOEC cultures

Since 1996, *S.* Brandenburg isolates have been typed by pulsed field gel electophoresis (PFGE). Those isolates showing the same DNA fingerprint resulting from digestion with the restriction endonuclease *Xba*I as an epidemic isolate were assigned as profile 14 (Clark, et al., 2004). These isolates that share the same PFGE genotype are considered to originate from the same strain. In our *ex vivo* assays, there was no difference among the isolates 3062, 3684, and 4468 for adhesion, invasion and replication (Table 4.4). Similarly, in the *in vitro* assays, there was no difference among isolates 3062, 3684, 4468 for adhesion, and invasion, although isolate 4468 replicated more than isolates 3062 and 3684 (Table 4.8). Hence, those isolates with a similar profile 14 genotype are associated with a similar phenotype for adhesion, invasion and replication.

In contrast, isolate 4527 that had a DNA banding pattern of non-profile 14 strains (Baker, et al., 2007; Wong, et al., 2007; Wright, et al., 1998) invaded less than profile 14 isolates 3062, 3684 and 4468 in *ex vivo* and *in vitro* cultures of OOECs.

*Salmonella*-induced invasion involves many genes and a rather intimate interaction between the bacteria and the host cell (Ochman & Groisman, 1996). Bacteria-induced invasion is initiated by ligand-receptor interactions on the host cell surface and injected effectors into the host cell, inducing cytoskeletal rearrangements that result in passive entry of bacteria (Patel & Galan, 2005). The invasion process involves a series of actin cytoskeletal changes induced by the translocated effectors. In mammalian cells, major rearrangements of the actin cytoskeleton upon receptor stimulation or other stimulation are controlled by specific small guanosine triphosphate (GTP)–binding proteins, CDC42 and Rac1 (Guiney & Lesnick, 2004). At least

five effectors contribute to *Salmonella*-induced invasion. These proteins activate GTP-binding protein-dependent signalling cascades (CDC-42 and Rac1) to induce cytoskeleton rearrangements (Hardt, et al., 1998; Zhou, et al., 2001), mediate the recovery of cytoskeletal architecture of the host cells once internalization occurs (Fu & Galan, 1999) and regulate bundling of actin filaments and nucleation of actin polymers (Hayward & Koronaki, 1999). *Salmonella* strains deficient in any of those effectors may exhibit a varied cellular invasion phenotype (Zhou, et al., 2001). Decreased invasion potential was described with another non-profile 14 *S.* Brandenburg isolate (NZRM 96-0105) as compared to isolate 3684 in both *in vitro* intestinal cell culture (7 days) and *in vivo* gut loop preparation (Brandt, et al., 2008). Together with our results it suggests that invasion of epithelial cells from at least 2 sites (intestine and oviduct) is impaired in the non-profile 14 phenotype.

In addition, in the *ex vivo* (but not the *in vitro*) culture, replication of the profile 14 isolates was higher than the non-profile 14 isolate. The reason for this discrepancy could relate to the different populations of cells or changed functionally in those cells cultured over 7 days.

Following invasion, *Salmonella* survive within a modified phagosome known as the *Salmonella* containing vacuole (SCV) (Ibarra & Steele-Mortimer, 2009). Late stage SCV modifications (6 to 8 hrs after infection) include the formation of tubular membrane extensions known as *Salmonella*-induced filaments (Sifs) in which intracellular replication in some cell types can be found (Drecktrah, et al., 2008). About 16 T3SS-2 effectors contribute to numerous intracellular events, including SCV maturation, Sif formation, bacterial replication and the systemic spread of bacteria. The intracellular pathogen residing in a vacuole triggers the Sif formation within which replication then occurs (Drecktrah, et al., 2008; Ramsden, et al., 2007). Mutation of the gene of coding for those effectors may vary the replication phenotype of *Salmonella* isolates.

Although, there is no direct evidence to suggest that non-profile 14 isolates are less virulent than the profile 14 isolates, all *S.* Brandenburg isolates recorded from the recent epidemic from the South Island of New Zealand were typed as profile 14 (Clark, et al., 2004). The decreased invasion and replication (at least in *ex vivo*) of non-profile 14 isolate 4527 is consistent with the results of Brandt et al. (2008), and leads to the conclusion that virulence of *S.* Brandenburg is correlated with bacterial invasion and replication potential because invasion of ileal loops *in vivo* has been associated with virulence. Potential gene sequences that may be associated with pathogenicity between *S.* Brandenburg profile 14 (3684) and non-profile 14

(96-0105) isolates have been predicted based on a genomic subtraction hybridisation technique (Brandt, et al., 2008).

### 5.1.2 Infection differences between *S.* Brandenburg and *S.* Typhimurium in OOEC cultures

In the *ex vivo* assays, most of the *S.* Brandenburg isolates (3062, 3684, 4468-all profile 14) adhered to and replicated within OOECs more than *S.* Typhimurium isolate 1979 (Table 4.4). On the contrary, in the *in vitro* assay the *S.* Typhimurium isolate 1979 adhered to and replicated within OOECs more than most of the *S.* Brandenburg isolates (3062, 3684, and 4527) (Table 4.8). That is, the infection characteristics of *Salmonella* differed in *ex vivo* and *in vitro* OOEC cultures. As the cells for the *ex vivo* assay had been isolated and cultured for only 24 h, the morphology, function and polarity were likely to have been better maintained compared to the *in vitro* OOECs, and thus are more likely to reflect the *in vivo* situation.

Bacterial adherence is a very critical step for initiating the infection and successful invasion and replication is dependent on the direct contact with host cells. Fimbriae are the main factors responsible for *Salmonella* adhesion. Most members of the *Salmonella* display numerous fimbriae on their cell surface that are thought to modulate interaction between pathogens and host cells (Brandt, et al., 2008; Wilson, et al., 2000). Genomic analysis reveals that 15 operons are found to encode fimbriae (Wagner & Hensel, 2011). The distribution of fimbrial operons among *Salmonella* strains suggests a role for fimbriae in pathogenesis. Broadly distributed fimbrial operons may provide general adhesive functions such as for *S.* Typhimurium but fimbriae whose distribution is limited may provide specific functions required in virulence for example *S.* Brandenburg (Edwards, et al., 1999). Further genome or proteomic analysis of the studied *Salmonella* isolate will be necessary to describe any such differences. Whatever the molecular reasons for the difference, we propose that enhanced adhesion to and replication within cells from the reproductive tract may be the reason that the profile 14 *S.* Brandenburg strains are more pathogenic for pregnant sheep reproductive tract compared to *S.* Typhimurium.

### 5.1.3 Infection differences between *S.* Brandenburg in OOEC and BOEC cultures

The replicating ability of *S.* Brandenburg isolate 3684 within OOECs was better than in BOECs for both *ex vivo* and *in vitro* assays (Table 4.6 and 4.10). This suggests that there is a degree of host specificity at the cellular level that may account for the more severe disease seen in sheep compared to cattle. In New Zealand over the past fifteen years, infections due to *S.* Brandenburg have been found in sheep, and cattle. The fact is that *S.* Brandenburg in cattle

has only occurred from areas where disease has been prevalent in sheep. This can occur from contaminated water ways and probably back-backed gulls. Furthermore, the disease prevalence in cattle is relatively lower than sheep, especially in the reproductive tract (Clarke & Tomlinson, 2004). In this study, the increased replicative ability of *S.* Brandenburg in OOEC compared to BOEC may account for the virulence of the disease in sheep. As faecal shedding of carrier animals and oral ingestion are important in maintaining *S.* Brandenburg in sheep flocks, one alternative possibility is that *S.* Brandenburg might be maintained in numbers high enough to infect sheep but too low to infect cattle that are co-grazing or in close proximity (Baskerville & Dow, 1973; Gray, et al., 1995). That is the induction of infectious diarrhea in cattle is insufficient to maintain *S.* Brandenburg on a long-term basis within the bovine population. The similar case has also been found in other serotypes. For example, *S.* Dublin is able to establish a carrier state in adult animals, which acts as a continual source of infection for cattle. If *S.* Brandenburg is unable to establish its own carriage in cattle this may limit its maintenance within the bovine population (Paulin, et al., 2002).

In addition, *Salmonella*-induced filaments (Sifs) are the replication site of bacteria inside the cells and are very essential for *Salmonella* replication. Although all of the mechanisms of *Salmonella* replication remained unknown, Sifs and Sif formation is only produced in 50-80% of infected cells and in certain animal species (Beuzon, et al., 2000; Boucrot, et al., 2003). This may be another reason that S. Brandenburg replicated greater in sheep than cattle.

### 5.1.4 Infection differences between *S.* Brandenburg in OOEC and OIEC cultures

In the present study, the *S.* Brandenburg isolate 3684 adhered to OOECs at a similar level to OIECs but invaded and replicated in OOECs more than OIECs (Table 4.12). This should enable *S.* Brandenburg to induce more significant damages to the ovine oviduct and surrounding tissues than to intestines, all other factors remaining the same. The cellular or biochemical reasons that could explain the differences between the 2 cell types are unknown. Infection with serotypes Abortusovis and Brandenberg are similar in that they both cause abortion *in vivo* but the mechanism may be different because the latter invades ovine ileal loops greater than Abortusovis (McFarlane & Lantier, unpublished). Moreover, there are substantial differences between the pathology of *S.* Brandenburg and *S.* Abortusovis following field (Mearns, 2007) or experimental (Li, et al., 2005) infections. The former causes gastrointestinal pathology and abortion commonly followed by death in the pregnant ewe whereas the latter causes few systemic clinical signs aside from abortion (Wray & Wray, 2000).

Paulin et al. (2002) suggested that disease outcomes of *S.* enteric serotypes in calves are associated with the degree of bacterial dissemination from the intestinal lumen to the target tissues. This was supported by the findings of Uzzau et al. (2000), in sheep, where *S. enterica* serovars Abortusovis (host restricted), Dublin (host adapted), and Gallinarum (host restricted) were recovered in comparable numbers from the intestinal mucosa, but serovar Gallinarum was recovered in lower numbers than the other serovars from systemic sites, following oral challenge. The pattern of bacterial recovery from systemic sites following intravenous inoculation was similar. Note that these experiments were conducted in young animals and did not include samples from the reproductive tract. Recently, levels of intestinal invasion (ovine ligated ileal loop) with *S.* Brandenburg have been shown to be at least as high as *S.* Typhimurium in Coopworth (Brandt, et al., 2008) and INRA 401 sheep (McFarlane & Lantier, unpublished). Our results indicate that dissemination of *S.* Brandenburg is not the only potential reason for pathology in the reproductive tract. There is an inherent ability of cells in the oviduct to encourage a higher rate of invasion and replication of these bacteria. Clearly if this finding can be confirmed with *in vivo* experiments, then it explains many of the features relating to the organisms tropism to the reproductive tract.

Although the infection characteristics of *S.* Brandenburg in this study can be linked to disease outcome and severity using *in vitro* or *ex vivo* modelling, this model system cannot represent the complex interaction operation within entire animals. Indeed, the validity of cultured cells as a model of physiological function *in vivo* has frequently been criticized. It has been pointed out that cells do not express the correct *in vivo* phenotype due to the altered cell's microenvironment. The cell-cell and cell-matrix interactions are reduced as the cells lack the heterogeneity and three-dimensional architecture present *in vivo*, and many hormonal and nutritional stimuli are absent. This creates an environment that favours the spreading, migration and proliferation of unspecialized progenitor cells rather than the highly differentiated and functional cells found *in vivo* (Freshney, 2005).

Furthermore, a cell culture does not contain components of the immune system such as antibodies, phagocytes and cytokines which are playing very critical roles in both host protection and host-specificity. These components exert their effects on host-pathogen interaction through various intricate biological processes. Typically following *Salmonella* infection, innate immune responses are present. Many host cells produce reactive oxygen molecules, largely through the activity of the phagosome NADPH oxidase (Nox2) that is counteracted by a *Salmonella*-produced superoxide dismutase, SodCI, conferring protection (Pacello, et al., 2008). In sheep, NRAMP1 (natural resistance-associated macrophage protein

one or Slc11A1), a divalent metal-proton symporter found in macrophages, neutrophils and DCs (Nairz, et al., 2009) was shown to inhibit the intestinal dissemination of the serovars Brandenburg, Typhimurium and Abortusovis in experiments with ovine ligated loops (McFarlane & Lantier, unpublished). In addition, the acquired immune response is critical for protection *in vivo* with both humoral and cell mediated components (Moore, et al., 2003; Pier, et al., 2004). Therefore, infecting cultured cells with *Salmonella* may only partially explain the bacterial infecting characteristics, pathogenesis and host specificity.

## 5.2 Cell preparation and optimization of infection assays

In former studies, mechanical disaggregation has been used to separate cell clumps. For example, Lasfargues (1973) pressed the dissected tissue through a series of sieves where the mesh is gradually reduced in size. Alternatively, the cell aggregate was pushed through a syringe graduating from wide to narrow gauge needles (Rottmayer, et al., 2006). By this method acceptable cell morphology and polarity remained. However, mechanical disaggregation is labour consuming and the separated cells easily re-clump due to cell-cell adhesion. Cell-cell adhesion in tissue is mediated by a variety of homotypic, interacting glycopeptides. Some of them are calcium-dependent in extracellular matrixes which are sensitive to chelating agents such as EDTA. Some exist in the intercellular matrix and basement membrane and are protease sensitive and can be digested by enzymes such as glycanases (Freshney, 2005). Thus, compared with mechanical disaggregation, using enzymes to disaggregate OECs is less labour consuming and fewer re-clumping events occurred, which was consistent with our results of optimisation experiments. For the *ex vivo* assays, newly isolated OECs needed to be disaggregated more aggressively. A combination of DNase I, collagenase and trypsin-EDTA was used to hydrolyse the DNA released from dead cells, chelate the $Ca^{2+}$ in the mucosa or oviduct fluid and digest the collagen contributing to cell-cell adhesion on the surface. As multiple enzymatic treatment was more damaging, as detected by cell viability estimates, a balance was achieved between cell yield and quality (Reischl, et al., 1999).

Serum contains growth factors which promote cell proliferation and adherence factors and exhibit antitrypsin activity, all of which promote cell attachment (Bottenstein, et al., 1979). The most popular sera in use are bovine calf serum or fetal bovine serum (FBS) for more demanding cell lines and cloning (Hyvonen, et al., 1988). The optimal concentration of bovine calf serum or fetal bovine serum used in the culture of OECs and IECs was 10%, a widely accepted concentration. However, BOECs started to adhere to the flask 1-2 days later

than the ovine cells in 10% FBS media and a confluent monolayer was formed after 8-9 days. Hence in order to achieve similar cell growth in ovine and bovine comparative OECs for infection assays, the concentrations of FBS were increased to 20%. This result is consistent with the reports from Ryan (1979).

Trypan blue does not enter a viable cell but, it does traverse the membrane of a dead cell. Hence the exclusion of trypan blue was used as a measure of cell viability (Al-Qubaisi, et al., 2011). In this study, over 95% of the harvested cells from the oviduct were viable, and those that were dead were typically single cells rather than those in aggregates, in agreement with Rottmayer et al. (2006). Viability estimates were particularly important for the *ex vivo* assays, where if the percentage of viable cells was too low (<80%) following treatment with the various hydrolytic enzymes, the infection assay with *Salmonella* was not carried out as adhesion, invasion and replication characteristics of the *Salmonella* were likely to be affected (Paulin, et al., 2007).

Cytokeratins (CK) are intermediate filament proteins making up cytoplasmic cytoskeletal structures of epithelial cells. They have been used to characterize epithelial cell types since they were initially found almost 30 years ago (Karantza, 2011; Moll, 1993). Immunocytochemistry is a practical and specific method to determine cell characteristics and distinguish between epithelial cells and fibroblast contamination because the monoclonal antibody is highly specific for a particular epitope of each of the cell types. In this study, monoclonal antibodies to cytokeratin 8 and 18 were used to characterize the cultured cells (Abe & Hoshi, 1997; Reischl, et al., 1999; Rottmayer, et al., 2006). In 7 day-old BOECs, the keratin filaments form a typical and clear complex network which extends from the surface of the nucleus to the cell membrane. This result is consistent with another report (Walter, 1995). Unlike BOECs, ovine cells expressed a relatively darker image which may reflect the weaker signal from a partly reacting bovine primary antibody to cytokeratin as suggested by Abe and Hoshi (1997).

Multiplicity of infection (MOI) refers to the number of bacteria or virus that is added per cell during infection. Typically low MOI is used when multiple cycles of infection are required. Kusters et al. (1993) suggest that the number of microorganisms that adhere or invade is related to the MOI. When added at equal MOIs, there are no significant differences in adhesion and invasion between mid-logarithmic and stationary-phase bacteria. They also reported that an incubation time of 30 mins is adequate for adhesion and 2 h for invasion when using a MOI $\geq$ 10, using S. Typhimurium. In this study, the MOI varied from 20-40

which ensured that all cells were exposed to bacteria and the infection environment among the experiments were consistent.

In conclusion, in this study *S.* Brandenburg profile 14 isolates (3062, 3684 and 4468) share similar phenotypes in adhesion, invasion and replication all of which are greater than a non-profile 14 isolate. Secondly, the profile 14 isolates invaded more than *S.* Typhimurium. Thirdly, ovine oviduct cells allow replication of *S.* Brandenburg to a greater extent than bovine cells. Fourthly, *S.* Brandenburg invades and replicates within ovine oviduct cells more readily than ovine intestinal cells. These findings in general support the 4 hypotheses proposed and may account for the observation that *S.* Brandenburg preferentially affects the reproductive tract of sheep.

# Chapter 6

# Future research

This thesis reports on a study that was designed to test differential isolate infectivity, host specificity and tissue tropism of *S.* Brandenburg by using *ex vivo* and *in vitro* oviduct epithelial cells and intestinal epithelial cells from sheep and cattle. The study was undertaken to account for field observations of abortion in sheep, in New Zealand with the intention of providing an alternative direction for control of this disease. However our studies have limitations and further research is recommended for the future.

Firstly, all the experiments were carried out in *ex vivo* and *in vitro* model systems which may not thoroughly mimic what happens *in vivo*. The results from those models cannot represent the complex interaction of whole animals. Therefore, an *in vivo* infection assay is recommended as a follow up experiment based on the results from *ex vivo* and *in vitro* model systems. The ligated ileal loop model has been used successfully in the past to assess intestinal infectivity *in vivo* but a novel method will need to be developed to assess infectivity of the reproductive tract.

Secondly, it is well known that the microclimate (nutritional, immunological) in which the *Salmonella* reside is critical to their survival. Hence the ability to manipulate these factors in conjunction with *in vivo* experiment should be informative. This may mean using animal models that have been manipulated to this end, such as genetic (NRAMP1 +/-) or immunological (DC +/-) knockouts. Work with the former is in progress.

Thirdly, former studies have revealed that several genes resident in SPI-1 and SPI-2 are responsible for *Salmonella* virulence which is play important roles in their adhesion, invasion and replication. Thus a molecular study on epidemic-strain specific genes should be meaningful among *S.* Brandenburg and other *Salmonella* serovars with links to phenotypic characteristics *in vitro* or *in vivo*.

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# Appendix A

# Sources of material

| Material | Supplier | Catalog NO. |
|---|---|---|
| 24-well plate | NUNC | 143982 |
| 75 cm2 cell culture flask | NUNC | 156367 |
| 25 cm2 cell culture flask | NUNC | 156499 |
| Amphotericin B | GIBCO | 04195780 D |
| Anti-mouse IgG-FITC | Sigma | F0257 |
| Bovine serum albumin (BSA) | Sigma | A7906 |
| Collagen | BD | 354236 |
| Collagenase | Sigma | 17018-029 |
| Dithiothreitol | BDH | 441494N |
| DNase I | Sigma | H5025 |
| Dulbecco's Modified Eagle Medium (D-MEM) | GIBCO | 11995-040 |
| Dulbecco's phosphate-buffered saline (D-PBS) | GIBCO | 14040-133 |
| EDTA | BDH | 100935V |
| EGF | Sigma | G9641 |
| Foetal bovine serum (FBS) | GIBCO | 10091-148 |
| Formaldehyde | AnalaR | 10113 |
| Gentamycin (10mg/ml) | GIBCO | 15710-072 |
| Giemsa | Sigma | G9641 |
| Hanks balanced salt solution (HBS) | GIBCO | 14170-112 |
| Harris haematoxylin | BDH | 351945s |
| Hydrocortisone | GIBCO | H6909 |
| Insulin | GIBCO | 12585-014 |
| Luria-Bertani broth | Sigma | L3522 |
| Monoclonal anti-cytokeratin pan antibody produced in mouse | Sigma | C2562 |
| Penicillin/streptomycin, (10,000 units) | GIBCO | 15140-122 |
| Poly-l-lysine | Sigma | P2636 |
| RPMI 1640 | GIBCO | 23400-021 |
| Sodium deoxycholate | Sigma | D6750 |
| Trypan blue | GIBCO | 15250-061 |
| Trypsin-EDTA | GIBCO | 25200-056 |

# Appendix B

# Single *Salmonella* infection experiments

## B.1 *Salmonella* infection in 24 h-old OOEC cultures

### B.1.1 Assay (1)

**Cell counting: 4.95 log $_{10}$/ml; 15 µL of inoculum in 15mL of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -6 | 37 | 42 | 36 | 3.70E+09 | 4.20E+09 | 3.60E+09 |
| 1979 | 1 | -6 | 44 | 31 | 35 | 4.40E+09 | 3.10E+09 | 3.50E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | OD (600nm) | MOI (CFU/cell) |
| 4527 | 9.57 | 9.62 | 9.56 | 3.83E+09 | 9.58 | 0.04 | 0.382 | 43 |
| 1979 | 9.64 | 9.49 | 9.54 | 3.67E+09 | 9.56 | 0.08 | 0.349 | 41 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -3 | 55 | 56 | 57 | 5.50E+06 | 5.60E+06 | 5.70E+06 |
| 1979 | 1 | -3 | 53 | 51 | 58 | 5.30E+06 | 5.10E+06 | 5.80E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 4527 | 6.74 | 6.75 | 6.76 | 5.60E+06 | 6.75 | 0.01 | 62 | 1 |
| 1979 | 6.72 | 6.71 | 6.76 | 5.40E+06 | 6.73 | 0.03 | 60 | 1 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 4527 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 1979 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 4527 | 1 | -1 | 24 | 21 | 20 | 21666.7 | 4.34 | 4.34 | 0.04 |
| | 2 | -1 | 20 | 19 | 20 | 19666.7 | 4.29 | | |
| | 3 | -1 | 26 | 21 | 25 | 24000.0 | 4.38 | | |
| 1979 | 1 | -1 | 29 | 24 | 22 | 25000.0 | 4.40 | 4.34 | 0.06 |
| | 2 | -1 | 21 | 17 | 20 | 19333.3 | 4.29 | | |
| | 3 | -1 | 22 | 23 | 18 | 21000.0 | 4.32 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 4527 | 1 | neat | 35 | 31 | 26 | 3066.7 | 3.49 | 3.39 | 0.12 |
| | 2 | neat | 30 | 27 | 23 | 2666.7 | 3.43 | | |
| | 3 | neat | 20 | 18 | 16 | 1800.0 | 3.26 | | |
| 1979 | 1 | -1 | 11 | 18 | 14 | 14333.3 | 4.16 | 4.21 | 0.06 |
| | 2 | -1 | 10 | 17 | 21 | 16000.0 | 4.20 | | |
| | 3 | -1 | 23 | 14 | 19 | 18666.7 | 4.27 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 4527 | 1 | -1 | 33 | 48 | 43 | 41333.3 | 4.62 | 4.78 | 0.04 |
| | 2 | -1 | 40 | 30 | 33 | 34333.3 | 4.54 | | |
| | 3 | -1 | 31 | 39 | 42 | 37333.3 | 4.57 | | |
| 1979 | 1 | -2 | 12 | 13 | 20 | 150000.0 | 5.18 | 5.15 | 0.04 |
| | 2 | -2 | 15 | 10 | 13 | 126666.7 | 5.10 | | |
| | 3 | -2 | 13 | 14 | 17 | 146666.7 | 5.17 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

**Cell counting: 4.93 log $_{10}$/ml; 15 µL of inoculum in 15mL of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -5 | 63 | 54 | 55 | 6.30E+08 | 5.40E+08 | 5.50E+08 |
| 3062 | 1 | -5 | 45 | 50 | 42 | 4.50E+08 | 5.00E+08 | 4.20E+08 |
| 4468 | 1 | -5 | 63 | 65 | 60 | 6.30E+08 | 6.50E+08 | 6.00E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |

| | Log(CFU/ml Count) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | OD (600nm) | MOI (CFU/cell) |
| 3684 | 8.80 | 8.73 | 8.74 | 5.73E+08 | 8.76 | 0.04 | 0.331 | 34 |
| 3062 | 8.65 | 8.70 | 8.62 | 4.57E+08 | 8.66 | 0.04 | 0.32 | 27 |
| 4468 | 8.80 | 8.81 | 8.78 | 6.27E+08 | 8.80 | 0.02 | 0.285 | 37 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 73 | 79 | 75 | 7.30E+06 | 7.90E+06 | 7.50E+06 |
| 3062 | 1 | -3 | 84 | 76 | 71 | 8.40E+06 | 7.60E+06 | 7.10E+06 |
| 4468 | 1 | -3 | 48 | 56 | 55 | 4.80E+06 | 5.60E+06 | 5.50E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |

| | Log Count | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | MOI (CFU/cell) | Growth rate |
| 3684 | 6.86 | 6.90 | 6.88 | 7.57E+06 | 6.88 | 0.02 | 89 | 3 |
| 3062 | 6.92 | 6.88 | 6.85 | 7.70E+06 | 6.89 | 0.04 | 91 | 3 |
| 4468 | 6.68 | 6.75 | 6.74 | 5.30E+06 | 6.72 | 0.04 | 62 | 2 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 3062 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 4468 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 49 | 55 | 43 | 49000.0 | 4.69 | 4.65 | 0.03 |
| | 2 | -1 | 44 | 48 | 40 | 44000.0 | 4.64 | | |
| | 3 | -1 | 44 | 43 | 40 | 42333.3 | 4.63 | | |
| 3062 | 1 | -1 | 33 | 45 | 49 | 42333.3 | 4.63 | 4.61 | 0.06 |
| | 2 | -1 | 34 | 32 | 38 | 34666.7 | 4.54 | | |
| | 3 | -1 | 42 | 44 | 48 | 44666.7 | 4.65 | | |
| 4468 | 1 | -1 | 52 | 67 | 58 | 59000.0 | 4.77 | 4.67 | 0.11 |
| | 2 | -1 | 46 | 44 | 57 | 49000.0 | 4.69 | | |
| | 3 | -1 | 33 | 30 | 43 | 35333.3 | 4.55 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | neat | 42 | 46 | 41 | 4300.0 | 3.63 | 3.67 | 0.03 |
| | 2 | neat | 49 | 44 | 47 | 4666.7 | 3.67 | | |
| | 3 | neat | 47 | 54 | 50 | 5033.3 | 3.70 | | |
| 3062 | 1 | neat | 42 | 50 | 38 | 4333.3 | 3.64 | 3.66 | 0.04 |
| | 2 | neat | 59 | 38 | 55 | 5066.7 | 3.70 | | |
| | 3 | neat | 43 | 42 | 49 | 4466.7 | 3.65 | | |
| 4468 | 1 | neat | 54 | 66 | 63 | 6100.0 | 3.79 | 3.79 | 0.08 |
| | 2 | neat | 82 | 70 | 72 | 7466.7 | 3.87 | | |
| | 3 | neat | 55 | 49 | 50 | 5133.3 | 3.71 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -2 | 16 | 15 | 15 | 153333.3 | 5.19 | 5.25 | 0.06 |
| | 2 | -2 | 17 | 23 | 21 | 203333.3 | 5.31 | | |
| | 3 | -2 | 15 | 20 | 18 | 176666.7 | 5.25 | | |
| 3062 | 1 | -2 | 14 | 10 | 11 | 116666.7 | 5.07 | 5.31 | 0.23 |
| | 2 | -2 | 17 | 28 | 21 | 220000.0 | 5.34 | | |
| | 3 | -2 | 41 | 24 | 37 | 340000.0 | 5.53 | | |
| 4468 | 1 | -2 | 25 | 25 | 22 | 240000.0 | 5.38 | 5.52 | 0.15 |
| | 2 | -2 | 32 | 28 | 33 | 310000.0 | 5.49 | | |
| | 3 | -2 | 47 | 45 | 51 | 476666.7 | 5.68 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.1.2 Assay (2)

**Cell counting: 5.08 log $_{10}$ /ml; 15 µl of inoculum in 15ml of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -6 | 37 | 42 | 36 | 3.70E+09 | 4.20E+09 | 3.60E+09 |
| 1979 | 1 | -6 | 44 | 31 | 35 | 4.40E+09 | 3.10E+09 | 3.50E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | OD (600nm) | MOI(CFU/cell) |
| 4527 | 9.57 | 9.62 | 9.56 | 3.83E+09 | 9.58 | 0.04 | 0.382 | 32 |
| 1979 | 9.64 | 9.49 | 9.54 | 3.67E+09 | 9.56 | 0.08 | 0.349 | 31 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -3 | 76 | 73 | 75 | 7.60E+06 | 7.30E+06 | 7.50E+06 |
| 1979 | 1 | -3 | 84 | 87 | 86 | 8.40E+06 | 8.70E+06 | 8.60E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 4527 | 6.88 | 6.86 | 6.88 | 7.47E+06 | 6.87 | 0.01 | 63 | 2 |
| 1979 | 6.92 | 6.94 | 6.93 | 8.57E+06 | 6.93 | 0.01 | 71 | 2 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 4527 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 1979 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 4527 | 1 | -1 | 22 | 31 | 33 | 28666.7 | 4.46 | 4.50 | 0.04 |
| | 2 | -1 | 27 | 36 | 36 | 33000.0 | 4.52 | | |
| | 3 | -1 | 31 | 34 | 37 | 34000.0 | 4.53 | | |
| 1979 | 1 | -1 | 28 | 27 | 31 | 28666.7 | 4.46 | 4.48 | 0.05 |
| | 2 | -1 | 28 | 28 | 29 | 28333.3 | 4.45 | | |
| | 3 | -1 | 33 | 30 | 41 | 34666.7 | 4.54 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 4527 | 1 | neat | 11 | 10 | 10 | 1033.3 | 3.01 | 3.07 | 0.05 |
| | 2 | neat | 13 | 11 | 15 | 1300.0 | 3.11 | | |
| | 3 | neat | 13 | 11 | 12 | 1200.0 | 3.08 | | |
| 1979 | 1 | neat | 20 | 20 | 15 | 1833.3 | 3.26 | 3.35 | 0.11 |
| | 2 | neat | 21 | 20 | 23 | 2133.3 | 3.33 | | |
| | 3 | neat | 28 | 25 | 36 | 2966.7 | 3.47 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 4527 | 1 | -1 | 10 | 14 | 15 | 13000.0 | 4.11 | 4.22 | 0.02 |
| | 2 | -1 | 17 | 13 | 11 | 13666.7 | 4.14 | | |
| | 3 | -1 | 13 | 13 | 11 | 12333.3 | 4.09 | | |
| 1979 | 1 | -1 | 24 | 28 | 26 | 26000.0 | 4.41 | 4.46 | 0.04 |
| | 2 | -1 | 36 | 30 | 26 | 30666.7 | 4.49 | | |
| | 3 | -1 | 31 | 26 | 31 | 29333.3 | 4.47 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

**Cell counting: 4.95 log $_{10}$/ml; 15 µL of inoculum in 15mL of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 26 | 22 | 27 | 2.60E+09 | 2.20E+09 | 2.70E+09 |
| 3062 | 1 | -6 | 22 | 30 | 40 | 2.20E+09 | 3.00E+09 | 4.00E+09 |
| 4468 | 1 | -6 | 38 | 28 | 20 | 3.80E+09 | 2.80E+09 | 2.00E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | OD (600nm) | MOI (CFU/cell) |
| 3684 | 9.41 | 9.34 | 9.43 | 2.50E+09 | 9.40 | 0.05 | 0.354 | 28 |
| 3062 | 9.34 | 9.48 | 9.60 | 3.07E+09 | 9.49 | 0.13 | 0.284 | 34 |
| 4468 | 9.58 | 9.45 | 9.30 | 2.87E+09 | 9.46 | 0.14 | 0.349 | 31 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 66 | 63 | 62 | 6.60E+06 | 6.30E+06 | 6.20E+06 |
| 3062 | 1 | -3 | 76 | 73 | 79 | 7.60E+06 | 7.30E+06 | 7.90E+06 |
| 4468 | 1 | -3 | 74 | 69 | 75 | 7.40E+06 | 6.90E+06 | 7.50E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 3684 | 6.82 | 6.80 | 6.79 | 6.37E+06 | 6.80 | 0.01 | 71 | 3 |
| 3062 | 6.88 | 6.86 | 6.90 | 7.60E+06 | 6.88 | 0.02 | 84 | 2 |
| 4468 | 6.87 | 6.84 | 6.88 | 7.27E+06 | 6.86 | 0.02 | 81 | 3 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 3062 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 4468 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -2 | 12 | 16 | 13 | 136666.7 | 5.14 | 5.17 | 0.09 |
| | 2 | -2 | 18 | 17 | 21 | 186666.7 | 5.27 | | |
| | 3 | -2 | 11 | 13 | 14 | 126666.7 | 5.10 | | |
| 3062 | 1 | -1 | 41 | 42 | 44 | 42333.3 | 4.63 | 4.60 | 0.03 |
| | 2 | -1 | 45 | 39 | 41 | 41666.7 | 4.62 | | |
| | 3 | -1 | 38 | 33 | 40 | 37000.0 | 4.57 | | |
| 4468 | 1 | -1 | 40 | 43 | 47 | 43333.3 | 4.64 | 4.63 | 0.02 |
| | 2 | -1 | 42 | 41 | 41 | 41333.3 | 4.62 | | |
| | 3 | -1 | 51 | 39 | 43 | 44333.3 | 4.65 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | neat | 51 | 57 | 53 | 5366.7 | 3.73 | 3.67 | 0.06 |
| | 2 | neat | 50 | 43 | 52 | 4833.3 | 3.68 | | |
| | 3 | neat | 43 | 37 | 40 | 4000.0 | 3.60 | | |
| 3062 | 1 | neat | 51 | 40 | 45 | 4533.3 | 3.66 | 3.54 | 0.21 |
| | 2 | neat | 49 | 44 | 46 | 4633.3 | 3.67 | | |
| | 3 | neat | 20 | 17 | 22 | 1966.7 | 3.29 | | |
| 4468 | 1 | neat | 23 | 31 | 24 | 2600.0 | 3.41 | 3.50 | 0.07 |
| | 2 | neat | 31 | 35 | 40 | 3533.3 | 3.55 | | |
| | 3 | neat | 33 | 38 | 31 | 3400.0 | 3.53 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -2 | 27 | 25 | 15 | 223333.3 | 5.35 | 5.31 | 0.06 |
| | 2 | -2 | 26 | 24 | 36 | 286666.7 | 5.46 | | |
| | 3 | -2 | 25 | 27 | 29 | 270000.0 | 5.43 | | |
| 3062 | 1 | -2 | 13 | 18 | 10 | 136666.7 | 5.14 | 5.22 | 0.09 |
| | 2 | -2 | 19 | 16 | 14 | 163333.3 | 5.21 | | |
| | 3 | -2 | 23 | 20 | 18 | 203333.3 | 5.31 | | |
| 4468 | 1 | -2 | 22 | 23 | 24 | 230000.0 | 5.36 | 5.29 | 0.14 |
| | 2 | -2 | 11 | 12 | 18 | 136666.7 | 5.14 | | |
| | 3 | -2 | 24 | 23 | 26 | 243333.3 | 5.39 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.1.3 Assay (3)

**Cell counting: 4.9 log $_{10}$ /ml; 15 µl of inoculum in 15ml of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -6 | 34 | 42 | 26 | 3.40E+09 | 4.20E+09 | 2.60E+09 |
| 1979 | 1 | -6 | 56 | 63 | 71 | 5.60E+09 | 6.30E+09 | 7.10E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | OD (600nm) | MOI(CFU/cell) |
| 4527 | 9.53 | 9.62 | 9.41 | 3.40E+09 | 9.53 | 0.10 | 0.382 | 45 |
| 1979 | 9.75 | 9.80 | 9.85 | 6.33E+09 | 9.80 | 0.05 | 0.349 | 84 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -3 | 54 | 55 | 51 | 5.40E+06 | 5.50E+06 | 5.10E+06 |
| 1979 | 1 | -3 | 68 | 60 | 59 | 6.80E+06 | 6.00E+06 | 5.90E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 4527 | 6.73 | 6.74 | 6.71 | 5.33E+06 | 6.73 | 0.02 | 76 | 2 |
| 1979 | 6.83 | 6.78 | 6.77 | 6.23E+06 | 6.79 | 0.03 | 83 | 1 |

| Gentamicin control (GC） | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 4527 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 1979 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | |
| 4527 | 1 | -1 | 17 | 10 | 12 | 13000.0 | 4.11 | 4.08 | 0.05 |
| | 2 | -1 | 14 | 11 | 13 | 12666.7 | 4.10 | | |
| | 3 | -1 | 10 | 11 | 11 | 10666.7 | 4.03 | | |
| 1979 | 1 | -1 | 12 | 11 | 17 | 13333.3 | 4.12 | 3.83 | 0.25 |
| | 2 | neat | 45 | 54 | 52 | 5033.3 | 3.70 | | |
| | 3 | neat | 41 | 43 | 57 | 4700.0 | 3.67 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 4527 | 1 | neat | 12 | 11 | 13 | 1200.0 | 3.08 | 3.10 | 0.02 |
| | 2 | neat | 15 | 14 | 10 | 1300.0 | 3.11 | | |
| | 3 | neat | 10 | 12 | 16 | 1266.7 | 3.10 | | |
| 1979 | 1 | neat | 28 | 25 | 26 | 2633.3 | 3.42 | 3.37 | 0.05 |
| | 2 | neat | 19 | 25 | 23 | 2233.3 | 3.35 | | |
| | 3 | neat | 23 | 25 | 17 | 2166.7 | 3.34 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 4527 | 1 | neat | 18 | 17 | 10 | 1500.0 | 3.18 | 3.38 | 0.16 |
| | 2 | neat | 22 | 23 | 20 | 2166.7 | 3.34 | | |
| | 3 | neat | 31 | 30 | 31 | 3066.7 | 3.49 | | |
| 1979 | 1 | neat | 44 | 41 | 41 | 4200.0 | 3.62 | 3.66 | 0.05 |
| | 2 | neat | 45 | 43 | 40 | 4266.7 | 3.63 | | |
| | 3 | neat | 48 | 51 | 59 | 5266.7 | 3.72 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

**Cell counting: 4.93 log $_{10}$/ml; 15 µl of inoculum in 15ml of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 26 | 22 | 27 | 2.60E+09 | 2.20E+09 | 2.70E+09 |
| 3062 | 1 | -6 | 22 | 30 | 40 | 2.20E+09 | 3.00E+09 | 4.00E+09 |
| 4468 | 1 | -6 | 38 | 28 | 20 | 3.80E+09 | 2.80E+09 | 2.00E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |

| | Log(CFU/ml Count) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | OD (600nm) | MOI (CFU/cell) |
| 3684 | 9.41 | 9.34 | 9.43 | 2.50E+09 | 9.40 | 0.05 | 0.354 | 32 |
| 3062 | 9.34 | 9.48 | 9.60 | 3.07E+09 | 9.49 | 0.13 | 0.284 | 36 |
| 4468 | 9.58 | 9.45 | 9.30 | 2.87E+09 | 9.46 | 0.14 | 0.349 | 34 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 51 | 53 | 57 | 5.10E+06 | 5.30E+06 | 5.70E+06 |
| 3062 | 1 | -3 | 67 | 83 | 75 | 6.70E+06 | 8.30E+06 | 7.50E+06 |
| 4468 | 1 | -3 | 71 | 54 | 66 | 7.10E+06 | 5.40E+06 | 6.60E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |

| | Log(CFU/ml Count | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 3684 | 6.71 | 6.72 | 6.76 | 5.37E+06 | 6.73 | 0.02 | 63 | 2 |
| 3062 | 6.83 | 6.92 | 6.88 | 7.50E+06 | 6.88 | 0.05 | 88 | 2 |
| 4468 | 6.85 | 6.73 | 6.82 | 6.37E+06 | 6.80 | 0.06 | 75 | 2 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 3062 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 4468 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 46 | 42 | 37 | 41666.7 | 4.62 | 4.58 | 0.04 |
| | 2 | -1 | 34 | 35 | 36 | 35000.0 | 4.54 | | |
| | 3 | -1 | 33 | 37 | 41 | 37000.0 | 4.57 | | |
| 3062 | 1 | -1 | 27 | 23 | 30 | 26666.7 | 4.43 | 4.48 | 0.05 |
| | 2 | -1 | 30 | 36 | 32 | 32666.7 | 4.51 | | |
| | 3 | -1 | 35 | 27 | 33 | 31666.7 | 4.50 | | |
| 4468 | 1 | -1 | 38 | 30 | 31 | 33000.0 | 4.52 | 4.56 | 0.06 |
| | 2 | -1 | 49 | 40 | 38 | 42333.3 | 4.63 | | |
| | 3 | -1 | 33 | 31 | 42 | 35333.3 | 4.55 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | neat | 51 | 57 | 44 | 5066.7 | 3.70 | 3.68 | 0.03 |
| | 2 | neat | 46 | 50 | 43 | 4633.3 | 3.67 | | |
| | 3 | neat | 45 | 43 | 48 | 4533.3 | 3.66 | | |
| 3062 | 1 | neat | 38 | 39 | 43 | 4000.0 | 3.60 | 3.60 | 0.04 |
| | 2 | neat | 41 | 44 | 45 | 4333.3 | 3.64 | | |
| | 3 | neat | 30 | 37 | 39 | 3533.3 | 3.55 | | |
| 4468 | 1 | neat | 48 | 41 | 38 | 4233.3 | 3.63 | 3.65 | 0.04 |
| | 2 | neat | 57 | 43 | 46 | 4866.7 | 3.69 | | |
| | 3 | neat | 33 | 52 | 41 | 4200.0 | 3.62 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 27 | 30 | 28 | 28333.3 | 4.45 | 4.34 | 0.04 |
| | 2 | -1 | 21 | 25 | 26 | 24000.0 | 4.38 | | |
| | 3 | -1 | 24 | 29 | 30 | 27666.7 | 4.44 | | |
| 3062 | 1 | -1 | 15 | 16 | 15 | 15333.3 | 4.19 | 4.23 | 0.10 |
| | 2 | -1 | 11 | 13 | 19 | 14333.3 | 4.16 | | |
| | 3 | -1 | 26 | 21 | 20 | 22333.3 | 4.35 | | |
| 4468 | 1 | -1 | 18 | 13 | 16 | 15666.7 | 4.19 | 4.14 | 0.07 |
| | 2 | -1 | 13 | 11 | 10 | 11333.3 | 4.05 | | |
| | 3 | -1 | 17 | 12 | 15 | 14666.7 | 4.17 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.2 *S.* Brandenburg infection in 24 h-old OOEC and BOEC cultures

### B.2.1 Assay (1), OOEC cultures

**Cell counting: 5.16 log $_{10}$ /ml; 15 µl of inoculum in 15ml of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 43 | 36 | 37 | 4.30E+09 | 3.60E+09 | 3.70E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/m)l | Avg.log(CFU/ml) | Error | OD (600nm) | MOI(CFU/cell) |
| 3684 | 9.63 | 9.56 | 9.57 | 3.87E+09 | 9.59 | 0.04 | 0.315 | 27 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -2 | 53 | 58 | 61 | 5.30E+06 | 5.80E+06 | 6.10E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 3684 | 6.72 | 6.76 | 6.79 | 5.73E+06 | 6.76 | 0.03 | 40 | 1 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 60 | 51 | 53 | 54666.7 | 4.74 | 4.73 | 0.05 |
| | 2 | -1 | 57 | 63 | 59 | 59666.7 | 4.78 | | |
| | 3 | -1 | 47 | 48 | 48 | 47666.7 | 4.68 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | neat | 14 | 21 | 23 | 1933.3 | 3.29 | 3.39 | 0.14 |
| | 2 | neat | 41 | 33 | 33 | 3566.7 | 3.55 | | |
| | 3 | neat | 26 | 18 | 20 | 2133.3 | 3.33 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -2 | 15 | 13 | 17 | 150000.0 | 5.18 | 5.09 | 0.08 |
| | 2 | -2 | 11 | 10 | 10 | 103333.3 | 5.01 | | |
| | 3 | -2 | 12 | 14 | 10 | 120000.0 | 5.08 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.2.2 Assay (2), OOEC cultures

**Cell counting: 5.19 log $_{10}$/ml; 15 µl of inoculum in 15ml of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 43 | 36 | 37 | 4.30E+09 | 3.60E+09 | 3.70E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | OD (600nm) | MOI(CFU/cell) |
| 3684 | 9.63 | 9.56 | 9.57 | 3.87E+09 | 9.59 | 0.04 | 0.315 | 25 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 63 | 65 | 71 | 6.30E+06 | 6.50E+06 | 7.10E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 3684 | 6.80 | 6.81 | 6.85 | 6.63E+06 | 6.82 | 0.03 | 43 | 2 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 55 | 59 | 64 | 59333.3 | 4.77 | 4.74 | 0.06 |
| | 2 | -1 | 62 | 47 | 66 | 58333.3 | 4.77 | | |
| | 3 | -1 | 53 | 46 | 41 | 46666.7 | 4.67 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | neat | 21 | 23 | 18 | 2066.7 | 3.32 | 3.37 | 0.05 |
| | 2 | neat | 27 | 20 | 25 | 2400.0 | 3.38 | | |
| | 3 | neat | 22 | 28 | 26 | 2533.3 | 3.40 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -2 | 14 | 12 | 15 | 136666.7 | 5.14 | 5.12 | 0.07 |
| | 2 | -2 | 11 | 10 | 12 | 110000.0 | 5.04 | | |
| | 3 | -2 | 13 | 19 | 13 | 150000.0 | 5.18 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.2.3 Assay (3), OOEC cultures

**Cell counting: 5.04 log $_{10}$/ml; 15 µl of inoculum in 15ml of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 43 | 36 | 37 | 4.30E+09 | 3.60E+09 | 3.70E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg. log(CFU/ml) | Error | OD (600nm) | MOI(CFU/cell) |
| 3684 | 9.63 | 9.56 | 9.57 | 3.87E+09 | 9.59 | 0.04 | 0.315 | 35 |

| Media control | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -2 | 61 | 63 | 79 | 6.10E+06 | 6.30E+06 | 7.90E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.OCFU/ml) | Avg. log(CFU/ml) | Error | MOI | Growth rate |
| 3684 | 6.79 | 6.80 | 6.90 | 6.77E+06 | 6.83 | 0.06 | 62 | 2 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 62 | 67 | 68 | 65666.7 | 4.82 | 4.82 | 0.05 |
| | 2 | -1 | 74 | 71 | 75 | 73333.3 | 4.87 | | |
| | 3 | -1 | 61 | 59 | 57 | 59000.0 | 4.77 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | neat | 22 | 21 | 23 | 2200.0 | 3.34 | 3.41 | 0.07 |
| | 2 | neat | 31 | 32 | 27 | 3000.0 | 3.48 | | |
| | 3 | neat | 25 | 29 | 24 | 2600.0 | 3.41 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -2 | 21 | 21 | 25 | 223333.3 | 5.35 | 5.23 | 0.11 |
| | 2 | -2 | 11 | 14 | 17 | 140000.0 | 5.15 | | |
| | 3 | -2 | 13 | 15 | 19 | 156666.7 | 5.19 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.2.4 Assay (1), BOEC cultures

**Cell counting: 4.92 log $_{10}$/ml; 15 µl of inoculum in 15ml of DMEM; 20%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 20 | 18 | 16 | 2.00E+09 | 1.80E+09 | 1.60E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | OD (600nm) | MOI(CFU/cell) |
| 3684 | 9.30 | 9.26 | 9.20 | 1.80E+09 | 9.26 | 0.05 | 0.304 | 22 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 35 | 31 | 41 | 3.50E+06 | 3.10E+06 | 4.10E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 3684 | 6.54 | 6.49 | 6.61 | 3.57E+06 | 6.55 | 0.06 | 45 | 2 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 20 | 17 | 24 | 20333.3 | 4.31 | 4.41 | 0.09 |
| | 2 | -1 | 29 | 28 | 29 | 28666.7 | 4.46 | | |
| | 3 | -1 | 34 | 28 | 24 | 28666.7 | 4.46 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | neat | 25 | 27 | 23 | 2500.0 | 3.40 | 3.43 | 0.04 |
| | 2 | neat | 36 | 23 | 30 | 2966.7 | 3.47 | | |
| | 3 | neat | 27 | 25 | 26 | 2600.0 | 3.41 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 25 | 19 | 21 | 21666.7 | 4.34 | 4.30 | 0.07 |
| | 2 | -1 | 18 | 14 | 18 | 16666.7 | 4.22 | | |
| | 3 | -1 | 19 | 22 | 24 | 21666.7 | 4.34 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.2.5 Assay (2), BOEC cultures

**Cell counting : 5.2 log $_{10}$ /ml; 15 µL of inoculum in 15mL of DMEM; 20%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 57 | 63 | 49 | 5.70E+09 | 6.30E+09 | 4.90E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | OD (600nm) | MOI(CFU/cell) |
| 3684 | 9.76 | 9.80 | 9.69 | 5.63E+09 | 9.75 | 0.05 | 0.353 | 36 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 44 | 54 | 51 | 4.40E+06 | 5.40E+06 | 5.10E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 3684 | 6.64 | 6.73 | 6.71 | 4.97E+06 | 6.70 | 0.05 | 32 | 1 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 56 | 53 | 51 | 53333.3 | 4.73 | 4.73 | 0.05 |
| | 2 | -1 | 57 | 63 | 65 | 61666.7 | 4.79 | | |
| | 3 | -1 | 44 | 48 | 53 | 48333.3 | 4.68 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain No. | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | neat | 68 | 62 | 63 | 6433.3 | 3.81 | 3.95 | 0.27 |
| | 2 | neat | 59 | 55 | 66 | 6000.0 | 3.78 | | |
| | 3 | -1 | 15 | 16 | 23 | 18000.0 | 4.26 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain No. | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 61 | 73 | 61 | 65000.0 | 4.81 | 4.76 | 0.05 |
| | 2 | -1 | 50 | 53 | 56 | 53000.0 | 4.72 | | |
| | 3 | -1 | 58 | 52 | 57 | 55666.7 | 4.75 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.2.6 Assay (3), BOEC cultures

**Cell counting: 5.04 log $_{10}$ /ml; 15 µL of inoculum in 15mL of DMEM; 20%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 45 | 42 | 41 | 4.50E+09 | 4.20E+09 | 4.10E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |

| Log(CFU/ml Count) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | OD (600nm) | MOI (CFU/cell) |
| 3684 | 9.65 | 9.62 | 9.61 | 4.27E+09 | 9.63 | 0.02 | 0.295 | 39 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 53 | 51 | 62 | 5.30E+06 | 5.10E+06 | 6.20E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |

| Log(CFU/ml Count) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 3684 | 6.72 | 6.71 | 6.79 | 5.53E+06 | 6.74 | 0.04 | 50 | 1 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -1 | 20 | 15 | 20 | 18333.3 | 4.26 | 4.39 | 0.12 |
| | 2 | -1 | 20 | 26 | 33 | 26333.3 | 4.42 | | |
| | 3 | -1 | 28 | 31 | 36 | 31666.7 | 4.50 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | neat | 38 | 26 | 36 | 3333.3 | 3.52 | 3.45 | 0.08 |
| | 2 | neat | 21 | 26 | 39 | 2866.7 | 3.46 | | |
| | 3 | neat | 23 | 23 | 24 | 2333.3 | 3.37 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | neat | 54 | 59 | 51 | 5466.7 | 3.74 | 3.75 | 0.06 |
| | 2 | neat | 63 | 66 | 65 | 6466.7 | 3.81 | | |
| | 3 | neat | 48 | 44 | 57 | 4966.7 | 3.70 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.3 *Salmonella* infection in 7 day-old OOEC cultures

### B.3.1 Assay (1)

**Cells count: 5.02 log $_{10}$/mL; 30 µL of inoculum in 15mL of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -5 | 53 | 41 | 48 | 5.30E+08 | 4.10E+08 | 4.80E+08 |
| 1979 | 1 | -5 | 40 | 31 | 47 | 4.00E+08 | 3.10E+08 | 4.70E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg. log(CFU/mL) | Error | DO (600nm) | MOI |
| 4527 | 8.72 | 8.61 | 8.68 | 4.73.E+08 | 8.67 | 0.06 | 0.352 | 30 |
| 1979 | 8.60 | 8.49 | 8.67 | 3.93.E+08 | 8.59 | 0.09 | 0.350 | 22 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -4 | 24 | 22 | 18 | 2.40E+07 | 2.20E+07 | 1.80E+07 |
| 1979 | 1 | -4 | 17 | 14 | 11 | 1.70E+07 | 1.40E+07 | 1.10E+07 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | MOI | Growth rate |
| 4527 | 7.38 | 7.34 | 7.26 | 2.13.E+07 | 7.33 | 0.06 | 201 | 7 |
| 1979 | 7.23 | 7.15 | 7.04 | 1.40.E+07 | 7.14 | 0.09 | 133 | 6 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 4527 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 1979 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 4527 | 1 | -2 | 41 | 33 | 28 | 340000 | 5.53 | 5.53 | 0.01 |
| | 2 | -2 | 35 | 32 | 33 | 333333 | 5.52 | | |
| | 3 | -2 | 28 | 37 | 39 | 346667 | 5.54 | | |
| 1979 | 1 | -2 | 57 | 66 | 65 | 626667 | 5.80 | 5.79 | 0.01 |
| | 2 | -2 | 56 | 59 | 63 | 593333 | 5.77 | | |
| | 3 | -2 | 56 | 59 | 68 | 610000 | 5.79 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 4527 | 1 | -1 | 39 | 38 | 38 | 38333 | 4.58 | 4.56 | 0.07 |
| | 2 | -1 | 30 | 29 | 33 | 30667 | 4.49 | | |
| | 3 | -1 | 38 | 40 | 46 | 41333 | 4.62 | | |
| 1979 | 1 | -2 | 18 | 16 | 15 | 163333 | 5.21 | 5.22 | 0.01 |
| | 2 | -2 | 16 | 21 | 12 | 163333 | 5.21 | | |
| | 3 | -2 | 20 | 17 | 13 | 166667 | 5.22 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 4527 | 1 | -2 | 73 | 81 | 84 | 793333 | 5.90 | 5.88 | 0.02 |
| | 2 | -2 | 75 | 77 | 70 | 740000 | 5.87 | | |
| | 3 | -2 | 81 | 77 | 68 | 753333 | 5.88 | | |
| 1979 | 1 | -3 | 17 | 17 | 13 | 1566667 | 6.19 | 6.20 | 0.02 |
| | 2 | -3 | 18 | 18 | 10 | 1533333 | 6.19 | | |
| | 3 | -3 | 19 | 19 | 12 | 1666667 | 6.22 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

**Cell counting: 5.20 log $_{10}$/ml; 15 µL of inoculum in 15mL of DMEM; 10%FBS**

| inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 8 | 7 | 8 | 8.00E+08 | 7.00E+08 | 8.00E+08 |
| 3062 | 1 | -6 | 6 | 7 | 5 | 6.00E+08 | 7.00E+08 | 5.00E+08 |
| 4468 | 1 | -6 | 3 | 6 | 4 | 3.00E+08 | 6.00E+08 | 4.00E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg.log(CFU/ml) | Error | OD (600nm) | MOI(CFU/cell) |
| 3684 | 8.90 | 8.85 | 8.90 | 7.67E+08 | 8.88 | 0.03 | 0.391 | 24 |
| 3062 | 8.78 | 8.85 | 8.70 | 6.00E+08 | 8.78 | 0.07 | 0.371 | 19 |
| 4468 | 8.48 | 8.78 | 8.60 | 4.33E+08 | 8.64 | 0.15 | 0.327 | 14 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -4 | 9 | 10 | 7 | 9.00E+06 | 1.00E+07 | 7.00E+06 |
| 3062 | 1 | -4 | 7 | 5 | 5 | 7.00E+06 | 5.00E+06 | 5.00E+06 |
| 4468 | 1 | -4 | 6 | 4 | 6 | 6.00E+06 | 4.00E+06 | 6.00E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/ml | Avg. log(CFU/ml) | Error | MOI (CFU/cell) | Growth rate |
| 3684 | 6.95 | 7.00 | 6.85 | 8.67E+06 | 6.94 | 0.08 | 54 | 2 |
| 3062 | 6.85 | 6.70 | 6.70 | 5.67E+06 | 6.75 | 0.08 | 42 | 2 |
| 4468 | 6.78 | 6.60 | 6.78 | 5.33E+06 | 6.73 | 0.10 | 42 | 3 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 3062 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 4468 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -3 | 11 | 22 | 14 | 1566666.7 | 6.19 | 5.92 | 0.24 |
| | 2 | -3 | 6 | 5 | 8 | 633333.3 | 5.80 | | |
| | 3 | -3 | 5 | 2 | 10 | 566666.7 | 5.75 | | |
| 3062 | 1 | -3 | 3 | 8 | 5 | 533333.3 | 5.73 | 5.73 | 0.21 |
| | 2 | -3 | 6 | 2 | 2 | 333333.3 | 5.52 | | |
| | 3 | -3 | 7 | 12 | 7 | 866666.7 | 5.94 | | |
| 4468 | 1 | -3 | 1 | 4 | 1 | 200000.0 | 5.30 | 5.26 | 0.13 |
| | 2 | -3 | 2 | 1 | 1 | 133333.3 | 5.12 | | |
| | 3 | -3 | 3 | 2 | 2 | 233333.3 | 5.37 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -2 | 11 | 12 | 15 | 12666.7 | 4.10 | 4.19 | 0.09 |
| | 2 | -2 | 15 | 10 | 21 | 15333.3 | 4.19 | | |
| | 3 | -2 | 23 | 15 | 19 | 19000.0 | 4.28 | | |
| 3062 | 1 | -2 | 22 | 24 | 27 | 24333.3 | 4.39 | 4.27 | 0.10 |
| | 2 | -2 | 15 | 13 | 20 | 16000.0 | 4.20 | | |
| | 3 | -2 | 16 | 15 | 19 | 16666.7 | 4.22 | | |
| 4468 | 1 | -2 | 12 | 12 | 21 | 15000.0 | 4.18 | 4.22 | 0.11 |
| | 2 | -2 | 17 | 11 | 13 | 13666.7 | 4.14 | | |
| | 3 | -2 | 22 | 23 | 20 | 21666.7 | 4.34 | | |
| LB | 1 | 0 | 0 | 0 | 0 | | | | |
| | 2 | 0 | 0 | 0 | 0 | | | | |
| | 3 | 0 | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/ml | Log10 | Average | Error |
| 3684 | 1 | -3 | 2 | 3 | 6 | 366666.7 | 5.56 | 5.49 | 0.12 |
| | 2 | -3 | 3 | 2 | 5 | 333333.3 | 5.52 | | |
| | 3 | -3 | 4 | 5 | 8 | 566666.7 | 5.75 | | |
| 3062 | 1 | -3 | 2 | 4 | 1 | 233333.3 | 5.37 | 5.66 | 0.25 |
| | 2 | -3 | 5 | 6 | 7 | 600000 | 5.78 | | |
| | 3 | -3 | 7 | 6 | 7 | 666666.7 | 5.82 | | |
| 4468 | 1 | -3 | 14 | 15 | 15 | 1466666.7 | 6.17 | 6.14 | 0.04 |
| | 2 | -3 | 12 | 19 | 6 | 1233333.3 | 6.09 | | |
| | 3 | -3 | 14 | 16 | 13 | 1433333.3 | 6.16 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.3.2 Assay (2)

**Cells count: 5.0 log $_{10}$/mL; 30 µL of inoculum in 15mL of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -5 | 53 | 41 | 48 | 5.30E+08 | 4.10E+08 | 4.80E+08 |
| 1979 | 1 | -5 | 40 | 31 | 47 | 4.00E+08 | 3.10E+08 | 4.70E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | DO (600nm) | MOI |
| 4527 | 8.72 | 8.61 | 8.68 | 4.73.E+08 | 8.67 | 0.06 | 0.352 | 32 |
| 1979 | 8.60 | 8.49 | 8.67 | 3.93.E+08 | 8.59 | 0.09 | 0.350 | 26 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -4 | 25 | 27 | 23 | 2.50E+07 | 2.70E+07 | 2.30E+07 |
| 1979 | 1 | -4 | 20 | 15 | 11 | 2.00E+07 | 1.50E+07 | 1.10E+07 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | MOI | Growth rate |
| 4527 | 7.40 | 7.43 | 7.36 | 2.50.E+07 | 7.40 | 0.03 | 250 | 8 |
| 1979 | 7.30 | 7.18 | 7.04 | 1.53.E+07 | 7.17 | 0.13 | 153 | 6 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 4527 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 1979 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 4527 | 1 | -2 | 31 | 35 | 39 | 350000 | 5.54 | 5.41 | 0.12 |
| | 2 | -2 | 21 | 18 | 25 | 213333 | 5.33 | | |
| | 3 | -2 | 18 | 17 | 31 | 220000 | 5.34 | | |
| 1979 | 1 | -2 | 51 | 40 | 41 | 440000 | 5.64 | 5.61 | 0.06 |
| | 2 | -2 | 35 | 33 | 35 | 343333 | 5.54 | | |
| | 3 | -2 | 39 | 50 | 45 | 446667 | 5.65 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 4527 | 1 | -1 | 38 | 33 | 37 | 36000 | 4.56 | 4.57 | 0.05 |
| | 2 | -1 | 35 | 37 | 31 | 34333 | 4.54 | | |
| | 3 | -1 | 42 | 41 | 45 | 42667 | 4.63 | | |
| 1979 | 1 | -2 | 11 | 14 | 10 | 116667 | 5.07 | 5.04 | 0.03 |
| | 2 | -2 | 12 | 11 | 10 | 110000 | 5.04 | | |
| | 3 | -2 | 10 | 10 | 11 | 103333 | 5.01 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 4527 | 1 | -2 | 52 | 53 | 47 | 506667 | 5.70 | 5.76 | 0.08 |
| | 2 | -2 | 80 | 71 | 63 | 713333 | 5.85 | | |
| | 3 | -2 | 56 | 52 | 51 | 530000 | 5.72 | | |
| 1979 | 1 | -3 | 15 | 12 | 22 | 1633333 | 6.21 | 6.11 | 0.09 |
| | 2 | -3 | 13 | 10 | 12 | 1166667 | 6.07 | | |
| | 3 | -3 | 13 | 10 | 10 | 1100000 | 6.04 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

**Cells count: 5.18 log $_{10}$ /mL; 15 µL of inoculum in 15mL of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -5 | 50 | 48 | 56 | 5.00E+08 | 4.80E+08 | 5.60E+08 |
| 3062 | 1 | -5 | 51 | 43 | 45 | 5.10E+08 | 4.30E+08 | 4.50E+08 |
| 4468 | 1 | -5 | 40 | 40 | 39 | 4.00E+08 | 4.00E+08 | 3.90E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |

| | Log(CFU/ml Count) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | 1 | 2 | 3 | Avg. CFU/mL | Avg.log(CFU/mL) | Error | DO (600nm) | MOI (CFU/cell) |
| 3684 | 8.70 | 8.68 | 8.75 | 5.13.E+08 | 8.71 | 0.03 | 0.476 | 23 |
| 3062 | 8.71 | 8.63 | 8.65 | 4.63.E+08 | 8.66 | 0.04 | 0.358 | 21 |
| 4468 | 8.60 | 8.60 | 8.59 | 3.97.E+08 | 8.60 | 0.01 | 0.363 | 18 |

| Media control | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 57 | 63 | 60 | 5.70E+06 | 6.30E+06 | 6.00E+06 |
| 3062 | 1 | -3 | 70 | 63 | 66 | 7.00E+06 | 6.30E+06 | 6.60E+06 |
| 4468 | 1 | -3 | 49 | 54 | 58 | 4.90E+06 | 5.40E+06 | 5.80E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |

| | Log(CFU/ml Count) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | MOI (CFU/cell) | Growth rate |
| 3684 | 6.76 | 6.80 | 6.78 | 6.00.E+06 | 6.78 | 0.02 | 45 | 2 |
| 3062 | 6.85 | 6.80 | 6.82 | 6.63.E+06 | 6.82 | 0.02 | 45 | 2 |
| 4468 | 6.69 | 6.73 | 6.76 | 5.37.E+06 | 6.73 | 0.04 | 45 | 3 |

| Gentamicin (All) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 3062 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 4468 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 40 | 38 | 35 | 376667 | 5.58 | 5.55 | 0.03 |
| | 2 | -2 | 30 | 36 | 32 | 326667 | 5.51 | | |
| | 3 | -2 | 37 | 38 | 34 | 363333 | 5.56 | | |
| 3062 | 1 | -2 | 37 | 38 | 33 | 360000 | 5.56 | 5.58 | 0.03 |
| | 2 | -2 | 39 | 40 | 43 | 406667 | 5.61 | | |
| | 3 | -2 | 38 | 39 | 37 | 380000 | 5.58 | | |
| 4468 | 1 | -2 | 28 | 36 | 33 | 323333 | 5.51 | 5.49 | 0.05 |
| | 2 | -2 | 22 | 31 | 28 | 270000 | 5.43 | | |
| | 3 | -2 | 33 | 32 | 33 | 326667 | 5.51 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -1 | 46 | 53 | 55 | 51333 | 4.71 | 4.77 | 0.06 |
| | 2 | -1 | 54 | 58 | 60 | 57333 | 4.76 | | |
| | 3 | -1 | 68 | 65 | 70 | 67667 | 4.83 | | |
| 3062 | 1 | -1 | 68 | 67 | 63 | 66000 | 4.82 | 4.76 | 0.06 |
| | 2 | -1 | 57 | 53 | 51 | 53667 | 4.73 | | |
| | 3 | -1 | 48 | 49 | 60 | 52333 | 4.72 | | |
| 4468 | 1 | -1 | 32 | 34 | 40 | 35333 | 4.55 | 4.48 | 0.10 |
| | 2 | -1 | 24 | 22 | 25 | 23667 | 4.37 | | |
| | 3 | -1 | 32 | 32 | 37 | 33667 | 4.53 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 21 | 19 | 20 | 200000 | 5.30 | 5.36 | 0.06 |
| | 2 | -2 | 25 | 20 | 24 | 230000 | 5.36 | | |
| | 3 | -2 | 28 | 22 | 30 | 266667 | 5.43 | | |
| 3062 | 1 | -2 | 26 | 27 | 28 | 270000 | 5.43 | 5.43 | 0.01 |
| | 2 | -2 | 24 | 27 | 29 | 266667 | 5.43 | | |
| | 3 | -2 | 26 | 26 | 30 | 273333 | 5.44 | | |
| 4468 | 1 | -2 | 70 | 75 | 69 | 713333 | 5.85 | 5.81 | 0.04 |
| | 2 | -2 | 63 | 66 | 64 | 643333 | 5.81 | | |
| | 3 | -2 | 60 | 59 | 61 | 600000 | 5.78 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.3.3 Assay (3)

**Cells count: 5.0 log $_{10}$/mL; 30 µL of inoculum in 15mL of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -5 | 68 | 60 | 67 | 6.80E+08 | 6.00E+08 | 6.70E+08 |
| 1979 | 1 | -5 | 32 | 33 | 29 | 3.20E+08 | 3.30E+08 | 2.90E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | DO (600nm) | MOI |
| 4527 | 8.83 | 8.78 | 8.83 | 6.50.E+08 | 8.81 | 0.03 | 0.366 | 22 |
| 1979 | 8.51 | 8.52 | 8.46 | 3.13.E+08 | 8.50 | 0.03 | 0.316 | 10 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 4527 | 1 | -3 | 70 | 62 | 65 | 7.00E+06 | 6.20E+06 | 6.50E+06 |
| 1979 | 1 | -3 | 29 | 32 | 34 | 2.90E+06 | 3.20E+06 | 3.40E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | MOI | Growth rate |
| 4527 | 6.85 | 6.79 | 6.81 | 6.57.E+06 | 6.82 | 0.03 | 66 | 3 |
| 1979 | 6.46 | 6.51 | 6.53 | 3.17.E+06 | 6.50 | 0.03 | 32 | 3 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 4527 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 1979 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 4527 | 1 | -2 | 32 | 28 | 30 | 300000 | 5.48 | 5.44 | 0.10 |
| | 2 | -2 | 21 | 15 | 28 | 213333 | 5.33 | | |
| | 3 | -2 | 30 | 33 | 35 | 326667 | 5.51 | | |
| 1979 | 1 | -3 | 51 | 50 | 54 | 516667 | 5.71 | 5.74 | 0.04 |
| | 2 | -3 | 59 | 61 | 63 | 610000 | 5.79 | | |
| | 3 | -3 | 50 | 58 | 51 | 530000 | 5.72 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 4527 | 1 | -1 | 28 | 37 | 31 | 32000 | 4.51 | 4.55 | 0.09 |
| | 2 | -1 | 51 | 40 | 44 | 45000 | 4.65 | | |
| | 3 | -1 | 31 | 26 | 34 | 30333 | 4.48 | | |
| 1979 | 1 | -2 | 18 | 20 | 23 | 203333 | 5.31 | 5.38 | 0.07 |
| | 2 | -2 | 29 | 30 | 26 | 283333 | 5.45 | | |
| | 3 | -2 | 25 | 21 | 27 | 243333 | 5.39 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 4527 | 1 | -2 | 65 | 63 | 61 | 630000 | 5.80 | 5.82 | 0.02 |
| | 2 | -2 | 61 | 67 | 74 | 673333 | 5.83 | | |
| | 3 | -2 | 72 | 72 | 66 | 700000 | 5.85 | | |
| 1979 | 1 | -3 | 13 | 11 | 14 | 1266667 | 6.10 | 6.09 | 0.05 |
| | 2 | -3 | 12 | 15 | 14 | 1366667 | 6.14 | | |
| | 3 | -3 | 11 | 10 | 12 | 1100000 | 6.04 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

**Cells count: 5.02 log $_{10}$ /mL; 15 µL of inoculum in 25mL of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -5 | 44 | 46 | 41 | 4.40E+08 | 4.60E+08 | 4.10E+08 |
| 3062 | 1 | -5 | 57 | 53 | 55 | 5.70E+08 | 5.30E+08 | 5.50E+08 |
| 4468 | 1 | -5 | 15 | 15 | 18 | 1.50E+08 | 1.50E+08 | 1.80E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | DO (600nm) | MOI |
| 3684 | 8.64 | 8.66 | 8.61 | 4.37.E+08 | 8.64 | 0.03 | 0.280 | 17 |
| 3062 | 8.76 | 8.72 | 8.74 | 5.50.E+08 | 8.74 | 0.02 | 0.323 | 21 |
| 4468 | 8.18 | 8.18 | 8.26 | 1.60.E+08 | 8.20 | 0.05 | 0.205 | 6 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 25 | 22 | 28 | 2.50E+06 | 2.20E+06 | 2.80E+06 |
| 3062 | 1 | -3 | 32 | 23 | 38 | 3.20E+06 | 2.30E+06 | 3.80E+06 |
| 4468 | 1 | -3 | 13 | 21 | 17 | 1.30E+06 | 2.10E+06 | 1.70E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg. log(CFU/mL) | Error | MOI | Growth rate |
| 3684 | 6.40 | 6.34 | 6.45 | 2.50.E+06 | 6.40 | 0.05 | 24 | 1 |
| 3062 | 6.51 | 6.36 | 6.58 | 3.10.E+06 | 6.48 | 0.11 | 30 | 1 |
| 4468 | 6.11 | 6.32 | 6.23 | 1.70.E+06 | 6.22 | 0.10 | 11 | 2 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 3062 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 4468 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 67 | 73 | 65 | 683333 | 5.83 | 5.81 | 0.03 |
| | 2 | -2 | 64 | 61 | 70 | 650000 | 5.81 | | |
| | 3 | -2 | 63 | 59 | 60 | 606667 | 5.78 | | |
| 3062 | 1 | -2 | 39 | 64 | 43 | 486667 | 5.69 | 5.70 | 0.14 |
| | 2 | -2 | 37 | 33 | 40 | 366667 | 5.56 | | |
| | 3 | -2 | 73 | 71 | 69 | 710000 | 5.85 | | |
| 4468 | 1 | -2 | 51 | 58 | 53 | 540000 | 5.73 | 5.77 | 0.05 |
| | 2 | -2 | 51 | 57 | 68 | 586667 | 5.77 | | |
| | 3 | -2 | 63 | 64 | 73 | 666667 | 5.82 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 31 | 20 | 28 | 263333 | 5.42 | 5.44 | 0.02 |
| | 2 | -2 | 30 | 33 | 25 | 293333 | 5.47 | | |
| | 3 | -2 | 23 | 30 | 28 | 270000 | 5.43 | | |
| 3062 | 1 | -2 | 24 | 23 | 30 | 256667 | 5.41 | 5.36 | 0.05 |
| | 2 | -2 | 20 | 20 | 23 | 210000 | 5.32 | | |
| | 3 | -2 | 25 | 21 | 20 | 220000 | 5.34 | | |
| 4468 | 1 | -2 | 15 | 10 | 13 | 126667 | 5.10 | 5.13 | 0.05 |
| | 2 | -2 | 12 | 12 | 15 | 130000 | 5.11 | | |
| | 3 | -2 | 11 | 20 | 15 | 153333 | 5.19 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -3 | 23 | 12 | 11 | 1533333 | 6.19 | 6.29 | 0.13 |
| | 2 | -3 | 16 | 20 | 18 | 1800000 | 6.26 | | |
| | 3 | -3 | 23 | 33 | 27 | 2766667 | 6.44 | | |
| 3062 | 1 | -3 | 10 | 10 | 14 | 1133333 | 6.05 | 6.14 | 0.09 |
| | 2 | -3 | 16 | 17 | 18 | 1700000 | 6.23 | | |
| | 3 | -3 | 19 | 11 | 12 | 1400000 | 6.15 | | |
| 4468 | 1 | -3 | 38 | 41 | 36 | 3833333 | 6.58 | 6.54 | 0.08 |
| | 2 | -3 | 31 | 30 | 23 | 2800000 | 6.45 | | |
| | 3 | -3 | 44 | 38 | 35 | 3900000 | 6.59 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.4 *S.* Brandenburg infection in 7 day-old OOEC and BOEC cultures

### B.4.1 Assay (1), OOEC cultures

**Cells count: 5.08 log $_{10}$/mL; 30 µL of inoculum in 15mL of DMEM; 20%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 11 | 15 | 10 | 1.10E+09 | 1.50E+09 | 1.00E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.FU/mL | Avg.log(CFU/mL | Error | DO (600nm) | MOI |
| 3684 | 9.04 | 9.18 | 9.00 | 1.20.E+09 | 9.07 | 0.09 | 0.348 | 50 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -4 | 17 | 14 | 21 | 1.70E+07 | 1.40E+07 | 2.10E+07 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL | Error | MOI | Growth rate |
| 3684 | 7.23 | 7.15 | 7.32 | 1.73.E+07 | 7.23 | 0.09 | 144 | 3 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 44 | 35 | 38 | 390000 | 5.59 | 5.54 | 0.04 |
| | 2 | -2 | 30 | 33 | 37 | 333333 | 5.52 | | |
| | 3 | -2 | 38 | 31 | 30 | 330000 | 5.52 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 29 | 30 | 27 | 286667 | 5.46 | 5.44 | 0.03 |
| | 2 | -2 | 28 | 22 | 25 | 250000 | 5.40 | | |
| | 3 | -2 | 30 | 25 | 30 | 283333 | 5.45 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 31 | 34 | 38 | 343333 | 5.54 | 5.47 | 0.06 |
| | 2 | -2 | 30 | 28 | 22 | 266667 | 5.43 | | |
| | 3 | -2 | 24 | 27 | 31 | 273333 | 5.44 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.4.2 Assay (2), OOEC cultures

**Cells count: 5.08 log $_{10}$ /mL; 30 µL of inoculum in 15mL of DMEM; 20%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -5 | 53 | 58 | 55 | 5.30E+08 | 5.80E+08 | 5.50E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | DO (600nm) | MOI |
| 3684 | 8.72 | 8.76 | 8.74 | 5.53.E+08 | 8.74 | 0.02 | 0.370 | 23 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 37 | 31 | 35 | 3.70E+06 | 3.10E+06 | 3.50E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | MOI | Growth rate |
| 3684 | 6.57 | 6.49 | 6.54 | 3.43.E+06 | 6.53 | 0.04 | 29 | 1 |

| Gentamicin (All) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 30 | 37 | 35 | 340000 | 5.53 | 5.55 | 0.03 |
| | 2 | -2 | 40 | 30 | 32 | 340000 | 5.53 | | |
| | 3 | -2 | 41 | 33 | 43 | 390000 | 5.59 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 27 | 26 | 22 | 250000 | 5.40 | 5.39 | 0.05 |
| | 2 | -2 | 26 | 25 | 30 | 270000 | 5.43 | | |
| | 3 | -2 | 21 | 23 | 21 | 216667 | 5.34 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -3 | 67 | 68 | 58 | 6433333 | 6.81 | 6.51 | 0.34 |
| | 2 | -3 | 11 | 17 | 14 | 1400000 | 6.15 | | |
| | 3 | -3 | 35 | 38 | 40 | 3766667 | 6.58 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.4.3 Assay (3), OOEC cultures

**Cells count: 5.02 log $_{10}$/mL; 30 µL of inoculum in 15mL of DMEM; 20%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -5 | 53 | 58 | 55 | 5.30E+08 | 5.80E+08 | 5.50E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | DO (600nm) | MOI |
| 3684 | 8.72 | 8.76 | 8.74 | 5.53.E+08 | 8.74 | 0.02 | 0.370 | 26 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 30 | 37 | 35 | 3.00E+06 | 3.70E+06 | 3.50E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | MOI | Growth rate |
| 3684 | 6.48 | 6.57 | 6.54 | 3.40.E+06 | 6.53 | 0.05 | 32 | 1 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 44 | 40 | 37 | 403333 | 5.61 | 5.67 | 0.06 |
| | 2 | -2 | 55 | 52 | 55 | 540000 | 5.73 | | |
| | 3 | -2 | 45 | 50 | 48 | 476667 | 5.68 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 36 | 38 | 35 | 363333 | 5.56 | 5.50 | 0.05 |
| | 2 | -2 | 35 | 25 | 32 | 306667 | 5.49 | | |
| | 3 | -2 | 30 | 31 | 25 | 286667 | 5.46 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -3 | 31 | 36 | 38 | 3500000 | 6.54 | 6.43 | 0.13 |
| | 2 | -3 | 27 | 36 | 26 | 2966667 | 6.47 | | |
| | 3 | -3 | 16 | 20 | 22 | 1933333 | 6.29 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.4.4 Assay (1), BOEC cultures

**Cells count: 5.13 log 10 /mL; 30 µL of inoculum in 15mL of DMEM; 20%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 10 | 10 | 13 | 1.00E+09 | 1.00E+09 | 1.30E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | DO (600nm) | MOI |
| 3684 | 9.00 | 9.00 | 9.11 | 1.10.E+09 | 9.04 | 0.07 | 0.348 | 44 |

| Media control (MC） | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -4 | 17 | 14 | 18 | 1.70E+07 | 1.40E+07 | 1.80E+07 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | MOI | Growth rate |
| 3684 | 7.23 | 7.15 | 7.26 | 1.63.E+07 | 7.21 | 0.06 | 121 | 3 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 40 | 43 | 39 | 406667 | 5.61 | 5.56 | 0.04 |
| | 2 | -2 | 35 | 37 | 32 | 346667 | 5.54 | | |
| | 3 | -2 | 32 | 31 | 40 | 343333 | 5.54 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 19 | 15 | 20 | 180000 | 5.26 | 5.31 | 0.11 |
| | 2 | -2 | 30 | 24 | 29 | 276667 | 5.44 | | |
| | 3 | -2 | 20 | 17 | 15 | 173333 | 5.24 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 37 | 34 | 26 | 323333 | 5.51 | 5.47 | 0.08 |
| | 2 | -2 | 33 | 36 | 32 | 336667 | 5.53 | | |
| | 3 | -2 | 24 | 21 | 28 | 243333 | 5.39 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.4.5 Assay (2), BOEC cultures

**Cells count: 5.15 log $_{10}$ /mL; 15 µL of inoculum in 15mL of DMEM; 20%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 12 | 16 | 13 | 1.20E+09 | 1.60E+09 | 1.30E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | DO (600nm) | MOI |
| 3684 | 9.08 | 9.20 | 9.11 | 1.37.E+09 | 9.13 | 0.06 | 0.320 | 33 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -4 | 15 | 16 | 14 | 1.50E+07 | 1.60E+07 | 1.40E+07 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | MOI | Growth rate |
| 3684 | 7.18 | 7.20 | 7.15 | 1.50.E+07 | 7.18 | 0.03 | 107 | 3 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 53 | 63 | 57 | 576667 | 5.76 | 5.76 | 0.01 |
| | 2 | -2 | 56 | 59 | 61 | 586667 | 5.77 | | |
| | 3 | -2 | 51 | 62 | 58 | 570000 | 5.76 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 30 | 32 | 35 | 323333 | 5.51 | 5.56 | 0.04 |
| | 2 | -2 | 38 | 41 | 32 | 370000 | 5.57 | | |
| | 3 | -2 | 36 | 38 | 42 | 386667 | 5.59 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 62 | 61 | 60 | 610000 | 5.79 | 5.73 | 0.05 |
| | 2 | -2 | 52 | 58 | 46 | 520000 | 5.72 | | |
| | 3 | -2 | 55 | 50 | 44 | 496667 | 5.70 | | |
| LB | 1 | -1 | 3 | 0 | 0 | | | | |
| | 2 | -1 | 4 | 0 | 0 | | | | |
| | 3 | -1 | 6 | 0 | 0 | | | | |

## B.4.6 Assay (3), BOEC cultures

**Cells count: 5.30 log $_{10}$ /mL; 15 µL of inoculum in 25mL of DMEM; 20%FBS**

| Inocula (All) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 12 | 16 | 13 | 1.20E+09 | 1.60E+09 | 1.30E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | DO (600nm) | MOI |
| 3684 | 9.08 | 9.20 | 9.11 | 1.37.E+09 | 9.13 | 0.06 | 0.320 | 23 |

| Media (All) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -4 | 12 | 20 | 11 | 1.20E+07 | 2.00E+07 | 1.10E+07 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | MOI | Growth rate |
| 3684 | 7.08 | 7.30 | 7.04 | 1.43.E+07 | 7.14 | 0.14 | 72 | 3 |

| Gentamicin (All) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion(1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 53 | 61 | 64 | 593333 | 5.77 | 5.80 | 0.02 |
| | 2 | -2 | 60 | 63 | 69 | 640000 | 5.81 | | |
| | 3 | -2 | 60 | 67 | 71 | 660000 | 5.82 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 41 | 40 | 38 | 396667 | 5.60 | 5.61 | 0.01 |
| | 2 | -2 | 39 | 43 | 45 | 423333 | 5.63 | | |
| | 3 | -2 | 37 | 40 | 44 | 403333 | 5.61 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication(24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 58 | 46 | 49 | 510000 | 5.71 | 5.67 | 0.04 |
| | 2 | -2 | 44 | 41 | 45 | 433333 | 5.64 | | |
| | 3 | -2 | 45 | 47 | 43 | 450000 | 5.65 | | |
| LB | 1 | -1 | 0 | 0 | 0 | | | | |
| | 2 | -1 | 0 | 0 | 0 | | | | |
| | 3 | -1 | 0 | 0 | 0 | | | | |

## B.5 *S.* Brandenburg infection in 7 day-old OIEC cultures

### B.5.1 Assay (1)

**Cells count: 4.99 log $_{10}$ /mL; 15 µL of inoculum in 15mL of DMEM; 10%FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 31 | 25 | 30 | 3.10E+09 | 2.50E+09 | 3.00E+09 |
| 1979 | 1 | -6 | 11 | 15 | 10 | 1.10E+08 | 1.50E+08 | 1.00E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | DO (600nm) | MOI |
| 3684 | 9.49 | 9.40 | 9.48 | 2.87.E+09 | 9.46 | 0.05 | 0.302 | 29 |
| 1979 | 8.04 | 8.18 | 8.00 | 1.20.E+08 | 8.07 | 0.09 | 0.296 | 12 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 52 | 55 | 56 | 5.20E+06 | 5.50E+06 | 5.60E+06 |
| 1979 | 1 | -3 | 26 | 27 | 22 | 2.60E+06 | 2.70E+06 | 2.20E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml) | Avg.log(CFU/ml) | Error | MOI | Growth rate |
| 3684 | 6.72 | 6.74 | 6.75 | 5.43.E+06 | 6.73 | 0.02 | 55 | 2 |
| 1979 | 6.41 | 6.43 | 6.34 | 2.50.E+06 | 6.40 | 0.05 | 26 | 2 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain No. | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 1979 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Avg | Error |
| 3684 | 1 | -2 | 41 | 35 | 38 | 380000 | 5.58 | 5.59 | 0.01 |
| | 2 | -2 | 40 | 43 | 37 | 400000 | 5.60 | | |
| | 3 | -2 | 38 | 41 | 40 | 396667 | 5.60 | | |
| 1979 | 1 | -2 | 12 | 10 | 14 | 120000 | 5.08 | 5.26 | 0.16 |
| | 2 | -2 | 22 | 24 | 26 | 240000 | 5.38 | | |
| | 3 | -2 | 23 | 20 | 21 | 213333 | 5.33 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Avg | Error |
| 3684 | 1 | -1 | 19 | 20 | 27 | 22000 | 4.34 | 4.26 | 0.08 |
| | 2 | -1 | 18 | 12 | 15 | 15000 | 4.18 | | |
| | 3 | -1 | 20 | 15 | 20 | 18333 | 4.26 | | |
| 1979 | 1 | neat | 84 | 86 | 89 | 8633 | 3.94 | 3.93 | 0.01 |
| | 2 | neat | 84 | 80 | 87 | 8367 | 3.92 | | |
| | 3 | neat | 82 | 87 | 86 | 8500 | 3.93 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Avg | Error |
| 3684 | 1 | -2 | 21 | 24 | 18 | 210000 | 5.32 | 5.34 | 0.04 |
| | 2 | -2 | 27 | 24 | 22 | 243333 | 5.39 | | |
| | 3 | -2 | 14 | 17 | 31 | 206667 | 5.32 | | |
| 1979 | 1 | -2 | 10 | 10 | 11 | 103333 | 5.01 | 5.02 | 0.02 |
| | 2 | -2 | 10 | 11 | 12 | 110000 | 5.04 | | |
| | 3 | -2 | 11 | 10 | 10 | 103333 | 5.01 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.5.2 Assay (2)

**Cells count: 5.06 log $_{10}$/mL; 15 µL of inoculum in 15mL of DMEM; 10% FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 31 | 25 | 30 | 3.10E+09 | 2.50E+09 | 3.00E+09 |
| 1979 | 1 | -6 | 11 | 15 | 10 | 1.10E+08 | 1.50E+08 | 1.00E+08 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.(CFU/ml） | Avg.log(CFU/ml) | Error | DO (600nm) | MOI |
| 3684 | 9.49 | 9.40 | 9.48 | 2.87.E+09 | 9.46 | 0.05 | 0.3 | 25 |
| 1979 | 8.04 | 8.18 | 8.00 | 1.20.E+08 | 8.07 | 0.09 | 0.303 | 10 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 61 | 55 | 50 | 6.10E+06 | 5.50E+06 | 5.00E+06 |
| 1979 | 1 | -3 | 35 | 32 | 30 | 3.50E+06 | 3.20E+06 | 3.00E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg.CFU/mL | Avg.log(CFU/mL) | Error | MOI | Growth rate |
| 3684 | 6.79 | 6.74 | 6.70 | 5.53.E+06 | 6.74 | 0.04 | 48 | 2 |
| 1979 | 6.54 | 6.51 | 6.48 | 3.23.E+06 | 6.51 | 0.03 | 28 | 3 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 1979 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 44 | 45 | 48 | 456667 | 5.66 | 5.65 | 0.02 |
| | 2 | -2 | 40 | 43 | 47 | 433333 | 5.64 | | |
| | 3 | -2 | 48 | 51 | 40 | 463333 | 5.67 | | |
| 1979 | 1 | -2 | 32 | 33 | 32 | 323333 | 5.51 | 5.43 | 0.08 |
| | 2 | -2 | 21 | 25 | 20 | 220000 | 5.34 | | |
| | 3 | -2 | 30 | 30 | 22 | 273333 | 5.44 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -1 | 19 | 23 | 27 | 23000 | 4.36 | 4.35 | 0.02 |
| | 2 | -1 | 28 | 12 | 24 | 21333 | 4.33 | | |
| | 3 | -1 | 17 | 25 | 25 | 22333 | 4.35 | | |
| 1979 | 1 | -1 | 15 | 13 | 17 | 15000 | 4.18 | 4.13 | 0.08 |
| | 2 | -1 | 15 | 15 | 16 | 15333 | 4.19 | | |
| | 3 | -1 | 10 | 11 | 12 | 11000 | 4.04 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Average | Error |
| 3684 | 1 | -2 | 41 | 44 | 38 | 410000 | 5.61 | 5.59 | 0.02 |
| | 2 | -2 | 40 | 37 | 42 | 396667 | 5.60 | | |
| | 3 | -2 | 34 | 37 | 41 | 373333 | 5.57 | | |
| 1979 | 1 | -2 | 11 | 12 | 12 | 116667 | 5.07 | 5.06 | 0.04 |
| | 2 | -2 | 10 | 12 | 15 | 123333 | 5.09 | | |
| | 3 | -2 | 11 | 10 | 10 | 103333 | 5.01 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

## B.5.3 Assay (3)

**Cells count: 5.01 log $_{10}$ /mL; 15 µL of inoculum in 15mL of DMEM; 10% FBS**

| Inocula | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -6 | 31 | 25 | 30 | 3.10E+09 | 2.50E+09 | 3.00E+09 |
| 1979 | 1 | -6 | 11 | 15 | 10 | 1.10E+09 | 1.50E+09 | 1.00E+09 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg. (CFU/ml) | Avg.log(CFU/ml) | Error | DO (600nm) | MOI |
| 3684 | 9.49 | 9.40 | 9.48 | 2.87.E+09 | 9.46 | 0.05 | 0.302 | 28 |
| 1979 | 9.04 | 9.18 | 9.00 | 1.20.E+09 | 9.07 | 0.09 | 0.296 | 12 |

| Media control (MC) | | | Spot Count | | | CFU/ml | | |
|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | 1 | 2 | 3 |
| 3684 | 1 | -3 | 73 | 63 | 84 | 7.30E+06 | 6.30E+06 | 8.40E+06 |
| 1979 | 1 | -3 | 21 | 23 | 32 | 2.10E+06 | 2.30E+06 | 3.20E+06 |
| LB | 1 | neat | 0 | 0 | 0 | | | |
| | Log(CFU/ml Count) | | | | | | | |
| Strain | 1 | 2 | 3 | Avg. (CFU/ml) | Avg. log(CFU/ml) | Error | MOI | Growth rate |
| 3684 | 6.86 | 6.80 | 6.92 | 7.33.E+06 | 6.86 | 0.06 | 71 | 3 |
| 1979 | 6.32 | 6.36 | 6.51 | 2.53.E+06 | 6.40 | 0.10 | 25 | 2 |

| Gentamicin control (GC) | | | Spot Count | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | Average | Error |
| 3684 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| 1979 | 1 | neat | 0 | 0 | 0 | 0 | 0 |
| LB | 1 | neat | 0 | 0 | 0 | 0 | 0 |

| Adhesion (1 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Avg | Error |
| 3684 | 1 | -2 | 46 | 51 | 45 | 473333 | 5.68 | 5.65 | 0.03 |
| | 2 | -2 | 42 | 39 | 44 | 416667 | 5.62 | | |
| | 3 | -2 | 45 | 47 | 41 | 443333 | 5.65 | | |
| 1979 | 1 | -2 | 14 | 13 | 11 | 126667 | 5.10 | 5.18 | 0.10 |
| | 2 | -2 | 15 | 16 | 12 | 143333 | 5.16 | | |
| | 3 | -2 | 16 | 24 | 19 | 196667 | 5.29 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Invasion (2 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Avg | Error |
| 3684 | 1 | -1 | 24 | 28 | 27 | 26333 | 4.42 | 4.43 | 0.01 |
| | 2 | -1 | 33 | 25 | 25 | 27667 | 4.44 | | |
| | 3 | -1 | 23 | 25 | 30 | 26000 | 4.41 | | |
| 1979 | 1 | -1 | 10 | 11 | 12 | 11000 | 4.04 | 4.04 | 0.02 |
| | 2 | -1 | 12 | 11 | 11 | 11333 | 4.05 | | |
| | 3 | -1 | 10 | 10 | 11 | 10333 | 4.01 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

| Replication (24 h) | | | Spot Count | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Strain | Sample | Dilution | 1 | 2 | 3 | CFU/mL | Log10 | Avg | Error |
| 3684 | 1 | -2 | 21 | 24 | 28 | 243333 | 5.39 | 5.38 | 0.01 |
| | 2 | -2 | 20 | 28 | 23 | 236667 | 5.37 | | |
| | 3 | -2 | 25 | 27 | 21 | 243333 | 5.39 | | |
| 1979 | 1 | -2 | 20 | 22 | 24 | 220000 | 5.34 | 5.36 | 0.02 |
| | 2 | -2 | 28 | 21 | 22 | 236667 | 5.37 | | |
| | 3 | -2 | 28 | 23 | 17 | 226667 | 5.36 | | |
| LB | 1 | neat | 0 | 0 | 0 | | | | |
| | 2 | neat | 0 | 0 | 0 | | | | |
| | 3 | neat | 0 | 0 | 0 | | | | |

# Appendix C

# Analysis of the effects of *Salmonella* infection

The tables display the Wald statistic tests for null effect. These statistics are approximately distributed as an F distribution with degrees of freedom calculated from the stratum variances (See Guide to Genstat, 2009). Significant *P* values for Wald tests indicate that means are significantly different; particular pairs of means are tested with *t*-tests.

## C.1 Analysis of the effects of *Salmonella* infection in 24 h-old OOEC cultures [Genstat REML (Payne, et al., 2009)].

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f | F pr |
|---|---|---|---|---|---|
| Isolate | 42.95 | 4 | 10.74 | 8.0 | 0.003 |
| Time_h | 1458.11 | 2 | 729.05 | 350.0 | <0.001 |
| Isolate.time_h | 112.12 | 8 | 14.01 | 350.0 | <0.001 |

## C.2 Analysis of the effects of *S.* Brandenburg infection in 24 h-old OOEC and BOEC cultures

| Fixed term | Wald statistic | d.f. | Wald/d.f. | Chi pr |
|---|---|---|---|---|
| Isolate | 0.00 | 0 | * | * |
| Time_h | 1214.03 | 2 | 607.02 | <0.001 |
| Species | 90.41 | 1 | 90.41 | <0.001 |
| Isolate.time_h | 0.00 | 0 | * | * |
| Isolate.species | 0.00 | 0 | * | * |
| Time_h.species | 197.85 | 2 | 98.92 | <0.001 |
| Isolate.time_h.species | 0.00 | 0 | * | * |

## C.3 Analysis of the effects of *Salmonella* infection in 7 day-old OOEC cultures

| Fixed term | Wald statistic | n.d.f. | F statistic | d.d.f | F pr |
|---|---|---|---|---|---|
| Isolate | 26.48 | 4 | 6.62 | 8.0 | 0.012 |
| Time_h | 1593.09 | 2 | 796.55 | 350.0 | <0.001 |
| Isolate.time_h | 188.19 | 8 | 23.52 | 350.0 | <0.001 |

## C.4 Analysis of the effects of *S.* Brandenburg infection in 7 day-old OOEC and BOEC cultures

| Fixed term | Wald statistic | d.f. | Wald/d.f. | Chi pr |
|---|---|---|---|---|
| Isolate | 0.00 | 0 | * | * |
| Time_h | 79.16 | 2 | 39.58 | <0.001 |
| Species | 34.09 | 1 | 34.09 | <0.001 |
| Isolate.time_h | 0.00 | 0 | * | * |
| Isolate.species | 0.00 | 0 | * | * |
| Time_h.species | 104.96 | 2 | 52.48 | <0.001 |
| Isolate.time_h.species | 0.00 | 0 | * | * |

## C.5 Analysis of the effect of *S.* Brandenburg infection in 7 day-old OIEC and OOEC cultures

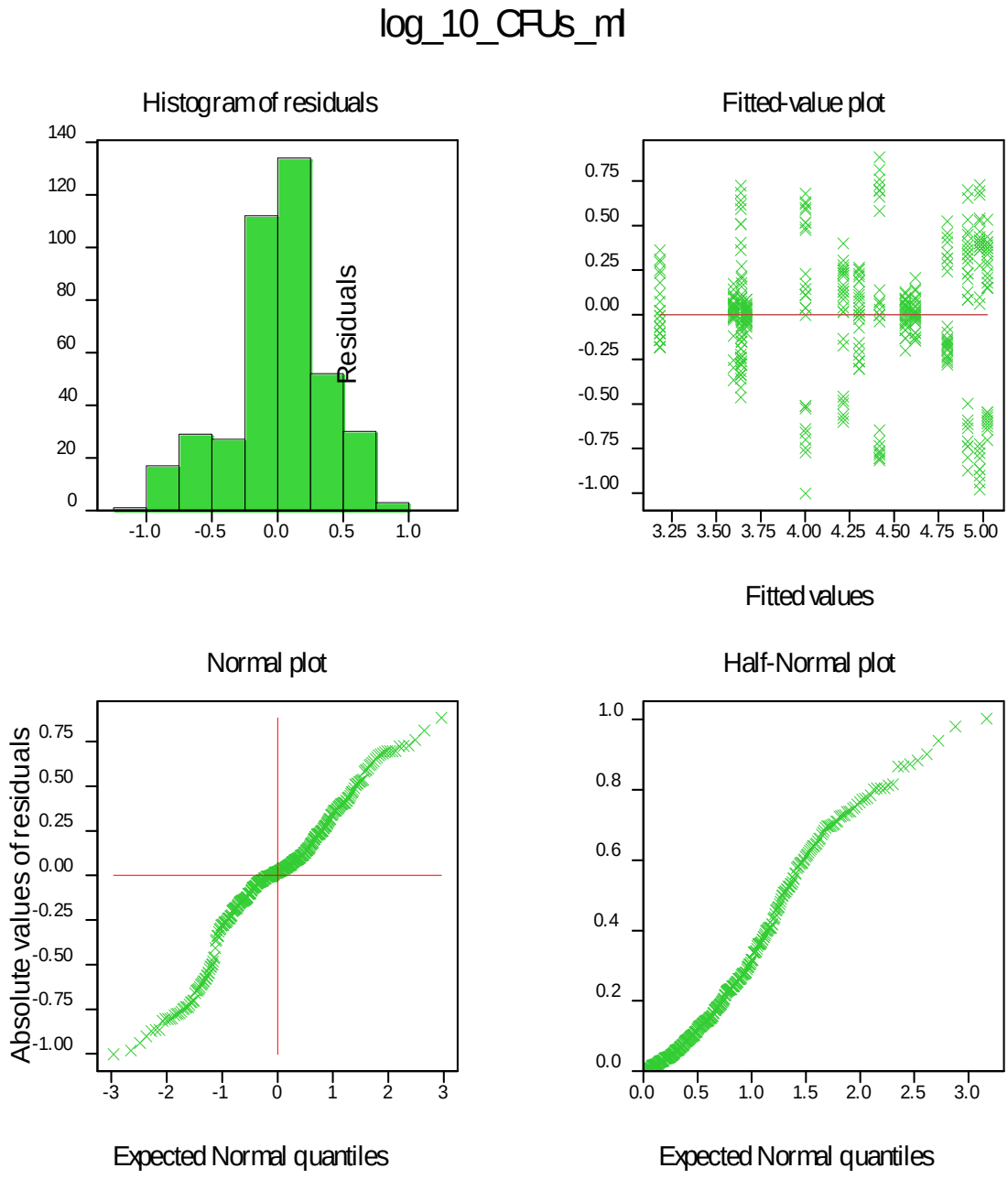
| Fixed term | Wald statistic | d.f. | Wald/d.f. | Chi pr |
|---|---|---|---|---|
| Isolate | 0.00 | 0 | * | * |
| Time_h | 586.71 | 2 | 293.35 | <0.001 |
| Cell_line | 130.20 | 1 | 130.20 | <0.001 |
| Isolate.time_h | 0.00 | 0 | * | * |
| Isolate.cell_line | 0.00 | 0 | * | * |
| Time_h.cell_line | 63.94 | 2 | 31.97 | <0.001 |
| Isolate.time_h.cell_line | 0.00 | 0 | * | * |

# Appendix D

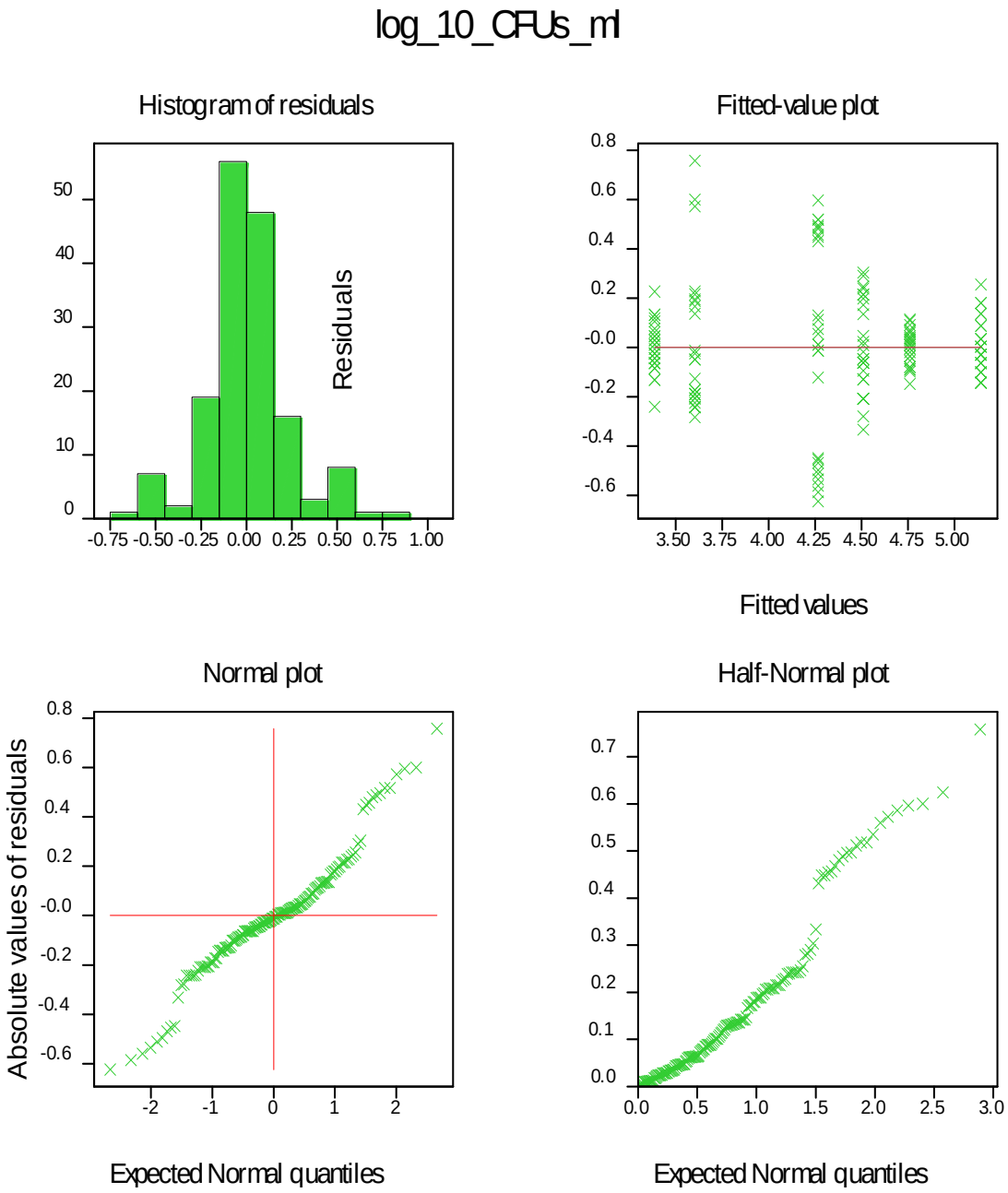
# Graphical tests of residuals

Histogram should display a normal distribution-bell-shaped curve in order to apply parametric analysis. The Fitted-value plot should show equal variation across the values of fitted values (usually means). The Normal plot (sometimes called a Q-Q plot) should show a straight line if the distribution is normal; likewise the Half-normal plot.

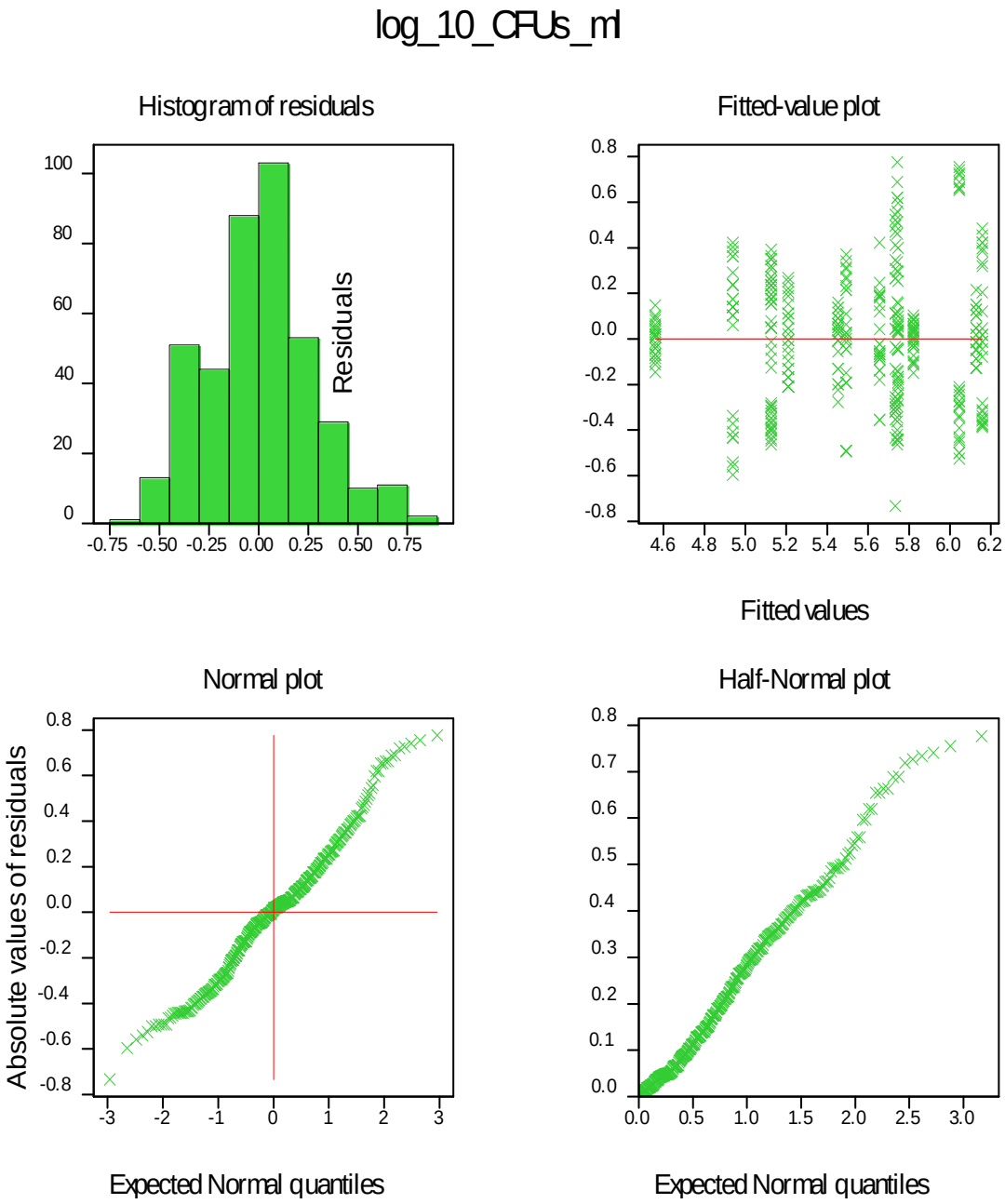
## D.1 *Salmonella* infection in 24 h-old OOEC cultures



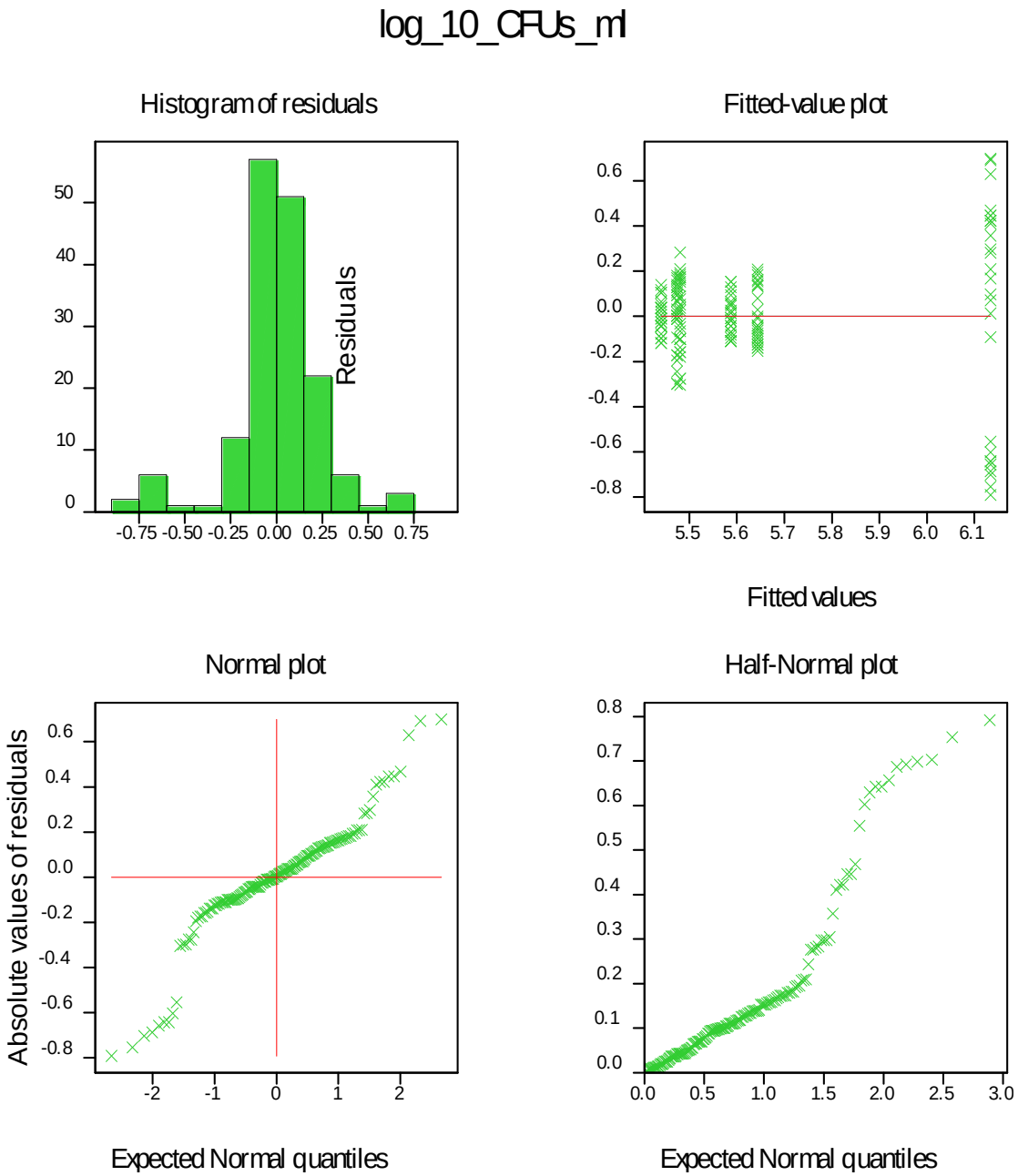
## D.2 *S.* Brandenburg infection in 24 h-old OOEC and BOEC cultures



## D.3 *Salmonella* infection in 7 day-old OOEC cultures



## D.4 *S.* Brandenburg infection in 7 day-old OOEC and BOEC cultures



## D.5 *S.* Brandenburg infection in 7 day-old OIEC and OOEC cultures